

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

B7

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 49/00, 51/04, C07F 13/00		A1	(11) International Publication Number: WO 95/28968
		(43) International Publication Date: 2 November 1995 (02.11.95)	
(21) International Application Number: PCT/US95/03763		(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).	
(22) International Filing Date: 24 March 1995 (24.03.95)			
(30) Priority Data: 231,599 22 April 1994 (22.04.94) US 397,469 1 March 1995 (01.03.95) US			
(71) Applicants: MONSANTO COMPANY [US/US]; 800 North Lindbergh Boulevard, St. Louis, MO 63167 (US). G.D. SEARLE & CO. [US/US]; 5200 Old Orchard Road, Skokie, IL 60077 (US).		Published With international search report.	
(72) Inventors: ALEXANDER, John, Charles; 1100 Pelham Road, Winnetka, IL 60093 (US). LENNON, Patrick, James; 7540 Wydown Boulevard, No. 3W, Clayton, MO 63105 (US). MODAK, Anil, Shrikrishna; 10355 Dover, No. 1217, Westminster, CO 80021 (US). NEUMANN, William, Lojda; 434 West Madison, Kirkwood, MO 63122 (US). RILEY, Dennis, Patrick; 800 Chancellor Heights Drive, Ballwin, MO 63011 (US). WEISS, Randy, Herman; 3074 Woodbridge Estates Drive, St. Louis, MO 63129 (US).			
(74) Agent: BOLDING, James, Clifton; Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO 63167 (US).			
(54) Title: DIAGNOSTIC IMAGE ANALYSIS WITH METAL COMPLEXES			
<p style="text-align: right;">(I)</p>			
(57) Abstract			
<p>The present invention is directed to complexes represented by formula (I) wherein R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉, R'₉, R₁₀, R₁₁, R₁₂, R₁₃, R₁₄, M, X, Y, Z and N are defined herein for use as contrast agents in diagnostic imaging.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

-1-

DIAGNOSTIC IMAGE ANALYSIS WITH METAL COMPLEXES

Background of the Invention

5

This invention relates to compounds effective as contrast agents in diagnostic imaging. In one aspect, this invention relates to magnetic resonance imaging (MRI) of human or non-human animal subjects using metal
10 complexes of substituted nitrogen-containing fifteen-membered macrocyclic ligands as contrast agents. In another aspect, this invention relates to manganese(II) complexes of substituted nitrogen-containing fifteen-membered macrocyclic ligands as MRI contrast agents.

15 X-rays have long been used to produce images of human and non-human animal tissue, e.g. the internal organs of a patient, the patient being positioned between a source of X-rays and a film sensitive to the rays. Where organs interfere with the passage of the
20 rays, the film is less exposed and the resulting developed film is indicative of the state of the organ.

More recently, nuclear magnetic resonance (NMR) has been developed as an imaging technique, i.e. MRI. MRI avoids the harmful effects sometimes attending X-ray
25 exposure. For improved imaging with X-rays, patients have been given enhancers prior to imaging, either orally or (parenterally). After a predetermined time interval for distribution of the enhancer through the patient, the image is taken. To obtain a good image it
30 is desirable that the time after the taking of enhancer be kept to a minimum. On the other hand there is a decrease in effectiveness with time, so desirably the decay should be relatively slow so as to provide a substantial time interval during which imaging can be
35 done.

-2-

substantial time interval during which imaging can be done.

In the NMR imaging process, protons in the water of the body relax via two mechanisms referred to as T_1 and T_2 . The rate at which the relaxation process occurs may be altered for some water molecules by giving values that contrast with the norm.

Compounds that enhance NMR images, referred to as contrast agents, are generally paramagnetic in nature. These may be organic free radicals or transition/lanthanide metals which have from one to seven unpaired electrons.

A necessary prerequisite of any ligand that binds a metal to form a contrast agent is that the resulting contrast agent be stable so as to prevent the loss of the metal and its subsequent accumulation in the body. Other considerations include an ability to reversibly bind water, which in turn increases its contrastability and decreases the dose level required. This ability is clearly important since the interaction between any two nuclear spins through space decreases at a rate equal to the reciprocal of the distance raised to the sixth power.

U.S. Pat. No. 4,647,447 discloses use of an NMR image enhancer consisting of the salt of an anion of a complexing acid and a paramagnetic metal anion. A preferred embodiment is the gadolinium chelate of diethylenetriaminepentaacetic acid (Gd DTPA), which is now commercially available from Nycomed Salutar, Inc. under the trade name Magnevist for use as an NMR contrast agent. From the data reported therein these appear to perform well. However, this compound is rapidly excreted by the kidneys, making the timing of the injection extremely critical. Furthermore, there is virtually no uptake by any solid organ, such as the heart, pancreas or liver.

-3-

However, while a number of gadolinium contrast agents are known, there remains the possibility that small amounts of free lanthanides are being released, by decomposition of the agent, into the body. Not being a naturally existing metal in the body, little is known about long term effects.

Other nitrogen-containing macrocyclic ligands have been suggested for use as NMR contrast agents. Jackels, S. C. et al, "Aqueous Proton NMR Relaxation Enhancements by Manganese(II) Macrocyclic Complexes: Structure-Relaxivity Relationships", *Inorg. Chem.*, 31, 234-239 (1992) discloses fifteen-membered nitrogen-containing ring complexes. However, these compounds suffer from being insufficiently stable and/or colored, and as such are inadequate for application as MRI contrast agents.

Therefore, it would be highly desirable to develop alternative contrast agents which avoid one or more of the aforementioned disadvantages.

It has now been discovered that metal complexes of substituted nitrogen-containing macrocyclic ligands which have increased kinetic, thermodynamic and oxidative stability, and which can be substituted to control lipophilicity, i.e. biodistribution, avoid the problems of the aforementioned contrast agents while providing good contrastability.

SUMMARY OF THE INVENTION

It is an object of the invention to provide magnetic resonance imaging (MRI) contrast agents having improved kinetic stability, i.e. the rate at which the paramagnetic metal dissociates from the metal complexes of the invention. It is a further object of the invention to provide MRI contrast agents in which the biodistribution of the contrast agents can be

-4-

controlled. It is yet a further object of the invention to provide MRI contrast agents having improved oxidative stability and improved hydrogen bonding. It is a still further object of the invention to provide metal
5 complexes which are useful as X-ray or ultrasound contrast agents, and which can be used in scintigraphy and radiotherapy.

According to the invention, a method of magnetic resonance imaging is provided which comprises
10 administering to a human or non-human animal subject a contrast medium comprising a physiologically compatible paramagnetic metal complex of the present invention and a non-toxic, pharmaceutically acceptable carrier, adjuvant or vehicle, and generating a magnetic resonance
15 image of at least a part of the subject.

Further according to the invention, a method of diagnostic imaging is provided which comprises administering to a human or non-human animal subject a diagnostic agent comprising a physiologically compatible
20 heavy metal complex of the present invention and a non-toxic, pharmaceutically acceptable carrier, adjuvant or vehicle, and generating an X-ray, ultrasound or scintigraphic image of at least a part of the subject.

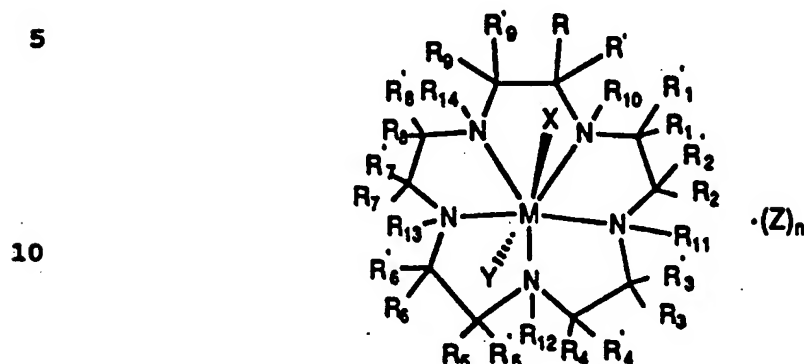
Further according to the invention, a method of
25 radiotherapy practiced on a human or non-human animal subject is provided which comprises administering to the subject a radioactive agent comprising a physiologically compatible radioactive metal complex of the present invention and a non-toxic, pharmaceutically acceptable
30 carrier, adjuvant or vehicle.

DETAILED DESCRIPTION OF THE INVENTION

The metal complexes of the invention used as MRI
35 contrast agents, as diagnostic agents in X-ray, ultra-

-5-

sound or scintigraphic image analysis, or as
radiotherapy agents are represented by the formula:

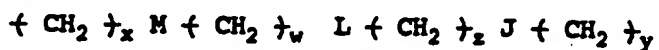


wherein R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉, and R'₉ independently are selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, cycloalkylcycloalkyl, cycloalkenylalkyl, alkylcycloalkyl, alkenylcycloalkyl, alkylcycloalkenyl, alkenylcycloalkenyl, heterocyclic, aryl and aralkyl radicals and radicals attached to the α-carbon of α-amino acids; or R₁ or R'₁ and R₂ or R'₂, R₃ or R'₃ and R₄ or R'₄, R₅ or R'₅ and R₆ or R'₆, R₇ or R'₇ and R₈ or R'₈, and R₉ or R'₉ and R or R' together with the carbon atoms to which they are attached independently form a saturated, partially saturated or unsaturated cyclic having 3 to 20 carbon atoms; R and R', R₁ and R'₁, R₂ and R'₂, R₃ and R'₃, R₄ and R'₄, R₅ and R'₅, R₆ and R'₆, R₇ and R'₇, R₈ and R'₈, and R₉ and R'₉ together with the carbon atoms to which they are attached independently form a saturated, partially saturated, or unsaturated ring structure having 3 to 20 carbon atoms; or one of R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉, R'₉, R₁₀,

-6-

R_{11} , R_{12} , R_{13} and R_{14} together with a different one of R , R' , R_1 , R'_1 , R_2 , R'_2 , R_3 , R'_3 , R_4 , R'_4 , R_5 , R'_5 , R_6 , R'_6 , R_7 , R'_7 , R_8 , R'_8 , R_9 , R'_9 , R_{10} , R_{11} , R_{12} , R_{13} and R_{14} which is attached to a different carbon or nitrogen atom in

5 the macrocyclic ligand may be bound to form a strap represented by the formula



wherein w , x , y and z independently are integers from 0 to 10, and M , L and J are independently selected from

10 the group consisting of alkyl, alkenyl, alkynyl, aryl, cycloalkyl, heteroaryl, alkaryl, alkheteroaryl, aza, amide, ammonium, thia, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphino, phosphonium, keto, ester, carbamate, urea, thiocarbonyl, borates, boranes,

15 boraza, silyl, siloxy, silaza and combinations thereof; and R_{10} , R_{11} , R_{12} , R_{13} and R_{14} independently are selected from the group consisting of hydrogen, alkyl, and alkyl substituted with $-\text{OR}_{15}$, $-\text{COOR}_{15}$, $-\text{CONR}_{15}\text{R}_{16}$ or $-\text{PO}_3\text{H}_2$ wherein R_{15} and R_{16} are independently hydrogen or alkyl;

20 and wherein at least two of R , R' , R_1 , R'_1 , R_2 , R'_2 , R_3 , R'_3 , R_4 , R'_4 , R_5 , R'_5 , R_6 , R'_6 , R_7 , R'_7 , R_8 , R'_8 , R_9 and R'_9 are other than hydrogen.

X , Y and Z represent suitable ligands or charge-neutralizing anions which are derived from any

25 monodentate or polydentate coordinating ligand or ligand system or the corresponding anion thereof (for example benzoic acid or benzoate anion, phenol or phenoxide anion, alcohol or alkoxide anion). X , Y and Z are independently selected from the group consisting of

30 halide, oxo, aquo, hydroxo, alcohol, phenol, dioxygen, peroxo, hydroperoxo, alkylperoxo, arylperoxo, ammonia, alkylamino, arylamino, heterocycloalkyl amino, heterocycloaryl amino, amine oxides, hydrazine, alkyl hydrazine, aryl hydrazine, nitric oxide, cyanide,

35 cyanate, thiocyanate, isocyanate, isothiocyanate, alkyl nitrile, aryl nitrile, alkyl isonitrile, aryl

-7-

- isonitrile, nitrate, nitrite, azido, alkyl sulfonic acid, aryl sulfonic acid, alkyl sulfoxide, aryl sulfoxide, alkyl aryl sulfoxide, alkyl sulfenic acid, aryl sulfenic acid, alkyl sulfinic acid, aryl sulfinic acid, alkyl thiol carboxylic acid, aryl thiol carboxylic acid, alkyl thiol thiocarboxylic acid, aryl thiol thiocarboxylic acid, alkyl carboxylic acid (such as acetic acid, trifluoroacetic acid, oxalic acid), aryl carboxylic acid (such as benzoic acid, phthalic acid),
- 10 urea, alkyl urea, aryl urea, alkyl aryl urea, thiourea, alkyl thiourea, aryl thiourea, alkyl aryl thiourea, sulfate, sulfite, bisulfate, bisulfite, thiosulfate, thiosulfite, hydrosulfite, alkyl phosphine, aryl phosphine, alkyl phosphine oxide, aryl phosphine oxide,
- 15 alkyl aryl phosphine oxide, alkyl phosphine sulfide, aryl phosphine sulfide, alkyl aryl phosphine sulfide, alkyl phosphonic acid, aryl phosphonic acid, alkyl phosphinic acid, aryl phosphinic acid, alkyl phosphinous acid, aryl phosphinous acid, phosphate, thiophosphate,
- 20 phosphite, pyrophosphite, triphosphate, hydrogen phosphate, dihydrogen phosphate, alkyl guanidino, aryl guanidino, alkyl aryl guanidino, alkyl carbamate, aryl carbamate, alkyl aryl carbamate, alkyl thiocarbamate, aryl thiocarbamate, alkyl aryl thiocarbamate, alkyl
- 25 dithiocarbamate, aryl dithiocarbamate, alkyl aryl dithiocarbamate, bicarbonate, carbonate, perchlorate, chlorate, chlorite, hypochlorite, perbromate, bromate, bromite, hypobromite, tetrahalomanganate, tetrafluoroborate, hexafluorophosphate,
- 30 hexafluoroantimonate, hypophosphite, iodate, periodate, metaborate, tetraaryl borate, tetra alkyl borate, tartrate, salicylate, succinate, citrate, ascorbate, saccharinate, amino acid, hydroxamic acid, thiotosylate, and anions of ion exchange resins, or
- 35 systems where one or more of X,Y and Z are independently attached to one or more of the "R" groups, wherein n is

an integer from 0 to 3. The preferred ligands from which X, Y and Z are selected include halide, organic acid, nitrate and bicarbonate anions.

The metal atoms or anions, M, which are suitable
5 for use in the complexes of the invention as MRI contrast agents are paramagnetic metals having atomic numbers 21-29, 42-44 and 57-71. The complexes for use as MRI contrast agents are those wherein the preferred metal is Eu, Gd, Dy, Ho, Cr, Mn or Fe, more preferably
10 Gd(III) or Mn(II), and most preferably Mn(II).

The metal atoms or anions, M, which are suitable for use in the complexes of the invention as X-ray or ultrasound contrast agents are heavy metals having atomic numbers 20-32, 42-44, 49 and 57-83. The
15 complexes for use as X-ray or ultrasound contrast agents are those wherein the preferred metal is a non-radioactive metal having atomic numbers 42-44, 49 and 57-83, and most preferably Gd, Dy or Yb.

The metal atoms or anions, M, of the complexes of
20 the invention which are suitable for use in scintigraphic and radiotherapy are radioactive metals of any conventional complexable radioactive metal isotope, preferably those having atomic numbers 20-32, 42-44, 49 and 57-83. In scintigraphy, the most preferred metals
25 are ^{99m}Tc or ^{111}In . In radiotherapy, the most preferred metals are ^{153}Sm , ^{67}Cu or ^{90}Y .

As utilized herein, the term "alkyl", alone or in combination, means a straight-chain or branched-chain alkyl radical containing from 1 to about 22 carbon
30 atoms, preferably from about 1 to about 18 carbon atoms, and most preferably from about 1 to about 12 carbon atoms. Examples of such radicals include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, iso-amyl,
35 hexyl, octyl, nonyl, decyl, dodecyl, tetradecyl, hexadecyl, octadecyl and eicosyl. The term "alkenyl",

-9-

alone or in combination, means an alkyl radical having one or more double bonds. Examples of such alkenyl radicals include, but are not limited to, ethenyl, propenyl, 1-butenyl, cis-2-butenyl, trans-2-butenyl, 5 iso-butylenyl, cis-2-pentenyl, trans-2-pentenyl, 3-methyl-1-butenyl, 2,3-dimethyl-2-butenyl, 1-pentenyl, 1-hexenyl, 1-octenyl, decenyl, dodecenyl, tetradecenyl, hexadecenyl, cis- and trans- 9-octadecenyl, 1,3-pentadienyl, 2,4-pentadienyl, 2,3-pentadienyl, 1,3-10 hexadienyl, 2,4-hexadienyl, 5,8,11,14-eicosatetraenyl, and 9,12,15-octadecatrienyl. The term "alkynyl", alone or in combination, means an alkyl radical having one or more triple bonds. Examples of such alkynyl groups include, but are not limited to, ethynyl, propynyl 15 (propargyl), 1-butyne, 1-octyne, 9-octadecyne, 1,3-pentadiyne, 2,4-pentadiyne, 1,3-hexadiyne, and 2,4-hexadiyne. The term "cycloalkyl", alone or in combination means a cycloalkyl radical containing from 3 to about 10, preferably from 3 to about 8, and most 20 preferably from 3 to about 6, carbon atoms. Examples of such cycloalkyl radicals include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and perhydronaphthyl. The term "cycloalkylalkyl" means an alkyl radical as defined 25 above which is substituted by a cycloalkyl radical as defined above. Examples of cycloalkylalkyl radicals include, but are not limited to, cyclohexylmethyl, cyclopentylmethyl, (4-isopropylcyclohexyl)methyl, (4-tert-butyl-cyclohexyl)methyl, 3-cyclohexylpropyl, 2-cyclo-30 hexylmethylpentyl, 3-cyclopentylmethylhexyl, 1-(4-neopentylcyclohexyl)methylhexyl, and 1-(4-isopropylcyclohexyl)methylheptyl. The term "cycloalkylcycloalkyl" means a cycloalkyl radical as defined above which is substituted by another cycloalkyl 35 radical as defined above. Examples of cycloalkylcycloalkyl radicals include, but are not

-10-

limited to, cyclohexylcyclopentyl and cyclohexylcyclohexyl. The term "cycloalkenyl", alone or in combination, means a cycloalkyl radical having one or more double bonds. Examples of cycloalkenyl radicals

5 include, but are not limited to, cyclopentenyl, cyclohexenyl, cyclooctenyl, cyclopentadienyl, cyclohexadienyl and cyclooctadienyl. The term "cycloalkenylalkyl" means an alkyl radical as defined

10 above which is substituted by a cycloalkenyl radical as defined above. Examples of cycloalkenylalkyl radicals include, but are not limited to, 2-cyclohexen-1-ylmethyl, 1-cyclopenten-1-ylmethyl, 2-(1-cyclohexen-1-yl)ethyl, 3-(1-cyclopenten-1-yl)propyl, 1-(1-cyclohexen-1-ylmethyl)pentyl, 1-(1-cyclopenten-1-yl)hexyl, 6-(1-

15 cyclohexen-1-yl)hexyl, 1-(1-cyclopenten-1-yl)nonyl and 1-(1-cyclohexen-1-yl)nonyl. The terms "alkylcycloalkyl" and "alkenylcycloalkyl" mean a cycloalkyl radical as defined above which is substituted by an alkyl or alkenyl radical as defined above. Examples of

20 alkylcycloalkyl and alkenylcycloalkyl radicals include, but are not limited to, 2-ethylcyclobutyl, 1-methylcyclopentyl, 1-hexylcyclopentyl, 1-methylcyclohexyl, 1-(9-octadecenyl)cyclopentyl and 1-(9-octadecenyl)cyclohexyl. The terms "alkylcycloalkenyl"

25 and "alkenylcycloalkenyl" means a cycloalkenyl radical as defined above which is substituted by an alkyl or alkenyl radical as defined above. Examples of alkylcycloalkenyl and alkenylcycloalkenyl radicals include, but are not limited to, 1-methyl-2-cyclopentyl,

30 1-hexyl-2-cyclopentenyl, 1-ethyl-2-cyclohexenyl, 1-butyl-2-cyclohexenyl, 1-(9-octadecenyl)-2-cyclohexenyl and 1-(2-pentenyl)-2-cyclohexenyl. The term "aryl", alone or in combination, means a phenyl or naphthyl radical which optionally carries one or more

35 substituents selected from alkyl, cycloalkyl, cycloalkenyl, phenyl, naphthyl, heterocycle, alkoxyaryl,

-11-

alkaryl, alkoxy, halogen, hydroxy, amine, cyano, nitro, alkylthio, phenoxy, ether, trifluoromethyl and the like, such as phenyl, p-tolyl, 4-methoxyphenyl, 4-(tert-butoxy)phenyl, 4-fluorophenyl, 4-chlorophenyl, 4-hydroxyphenyl, 1-naphthyl, 2-naphthyl, and the like.

The term "aralkyl", alone or in combination, means an alkyl or cycloalkyl radical as defined above in which one hydrogen atom is replaced by an aryl radical as defined above, such as benzyl, 2-phenylethyl, and the like.

The term "heterocyclic" means ring structures containing at least one other kind of atom, in addition to carbon, in the ring. The most common of the other kinds of atoms include nitrogen, oxygen and sulfur. Examples of heterocyclics include, but are not limited to, pyrrolidinyl, piperidyl, imidazolidinyl, tetrahydrofuryl, tetrahydrothienyl, furyl, thienyl, pyridyl, quinolyl, isoquinolyl, pyridazinyl, pyrazinyl, indolyl, imidazolyl, oxazolyl, thiazolyl, pyrazolyl, pyridinyl, benzoxadiazolyl, benzothiadiazolyl, triazolyl and tetrazolyl groups.

The term "saturated, partially saturated or unsaturated cyclic" means fused ring structures in which 2 carbons of the ring are also part of the fifteen-membered macrocyclic ligand. The ring structure can contain 3 to 20 carbon atoms, preferably 5 to 8 carbon atoms, and can also contain one or more other kinds of atoms in addition to carbon. The most common of the other kinds of atoms include nitrogen, oxygen and sulfur. The ring structure can also contain more than one ring.

The term "saturated, partially saturated or unsaturated ring structure" means a ring structure in which one carbon of the ring is also part of the fifteen-membered macrocyclic ligand. The ring structure can contain 3 to 20, preferably 5 to 8, carbon atoms and can also contain nitrogen, oxygen and/or sulfur atoms.

The term "organic acid anion" refers to carboxylic acid anions having from about 1 to about 18

-12-

carbon atoms. The term "halide" means chloride or bromide.

The overall charge-type of the complex can be varied from negative to positive by nitrogen or carbon substitution of the appropriate charged groups on the macrocyclic framework. While the manganese (II) complexes of the invention exist as monocations in methanol solution, the axial anions are labile and in vivo can rapidly exchange with endogenous charged or uncharged ligands. By considering the dispositive nature of the manganese (II) metal center, the overall charge on the complex can be adjusted as needed to enhance desired pharmaceutical properties such as osmolality, tissue distribution and non-target clearance. For example, if the complex carries only charge neutral functionality, such as N- or C-alkyl substitution, then the overall charge on the complex will be determined by the manganese center and will be positive. Multi-positive complexes are available via the incorporation of pendant cations such as protonated aminoalkyl groups. These types of complexes can bind to endogenous anions, anionic proteins, cell membranes, and the like. If two pendant anionic groups are attached, such as two carboxylates, phenolate, phosphonates, sulfonates and the like, the overall charge on the complex can be envisioned as zero. Alternatively, if three or more pendant anionic groups are attached, the an anionic complex will result. The pendant groups may be designed to axially chelate and formally displace the axial anions or they may be designed specifically to not chelate but retain a charge type.

The substituents on the complex of the invention, i.e. the "R" groups other than hydrogen, are those groups which result in complexes having improved stability, controlled lipophilicity, improved hydrogen bonding and greater rigidity of the macrocyclic ligand.

-13-

Regarding rigidity of the macrocycle, groups which rigidify the macrocycle result in improved stability and improved inner- and outer-sphere relaxation. Examples of groups which improve rigidity of the macrocycle include, but are not limited to, cycloalkyl groups e.g. trans-cyclohexano, and multiple alkyl groups, e.g. pentamethyl.

Regarding hydrogen bonding, groups that improve hydrogen bonding result in improved residence time of water to the metal complex by providing alternate binding sites. Examples of groups that improve hydrogen bonding include, but are not limited to, hydroxy alkyl, e.g. hydroxymethyl.

By varying the type and number of substituents, e.g. "R" groups which are other than hydrogen, the lipophilicity of the complexes can be controlled, i.e. the biodistribution of the complexes of the invention can be controlled, by preparing compounds which vary from hydrophilic to lipophilic. Therefore, the complexes of the invention can be targeted to various tissues or organs in the body by controlling the type and number of substituents.

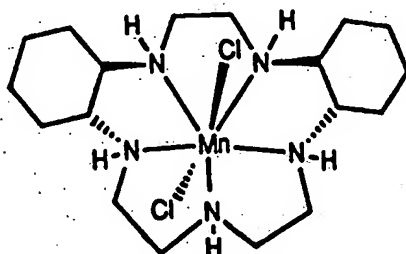
Kinetic stability of the metal complex is important because complexes which are not sufficiently kinetically stable dissociate and release free metal in the body. The kinetic stability, k_{diss} ($\text{M}^{-1}\text{sec}^{-1}$), can be controlled by varying the type and number of substituents which are other than hydrogen. The complexes of the invention have $k_{\text{diss}} \leq 1400 \text{ M}^{-1}\text{sec}^{-1}$, i.e. the complexes of the invention are at least twice as stable as the complex in which all R's are hydrogen. In addition, the type and number of substituents can be selected to give complexes which are at least 1000 times more kinetically stable than the complex in which all R's are hydrogen. Examples of groups that improve kinetic stability include, but are not limited to, cycloalkyl groups, e.g.

-14-

trans-cyclohexano, and multiple alkyl groups, e.g. penta- methyl. Oxidative stability of the metal complex is a particular problem for Mn complexes and is important because complexes which are not sufficiently
 5 oxidatively stable will go from Mn(II) to Mn(III). Since the Mn(III) complexes are colored, it is necessary to maintain the complexes in the Mn(II) form to have an effective contrast agent. By varying the type and number of substituents, the oxidative stability,
 10 $E_{1/2}(v)$, is controlled. It is generally desired to select the type of number of substituents such that $E_{1/2}$ is greater than about 0.7v.

The number of "R" groups attached to carbon atoms, i.e. $R-R_9$ and $R'-R_9'$, which are other than hydrogen is
 15 preferably at least 3, and more preferably at least 5.

One group of currently preferred compounds are those in which at least one of R_1 or R'_1 and R_2 or R'_2 , R_3 or R'_3 and R_4 or R'_4 , R_5 or R'_5 and R_6 or R'_6 , R_7 or R'_7 and R_8 or R'_8 , and R_9 or R'_9 and R or R' together
 20 with the carbon atoms to which they are attached form a saturated cyclic having 5 to 8 carbon atoms; and all of the remaining "R" groups are independently selected from hydrogen, alkyl, or alkyl substituted with $-OR_{15}$ or $-NR_{15}R_{16}$ wherein R_{15} and R_{16} are independently hydrogen or
 25 alkyl. The number of saturated cyclic rings can vary from one to 5, but is preferably at least 2, and the most preferred saturated cyclic has a ring size of 6 carbon atoms; i.e. is a cyclohexano group. An example of such a compound is represented by the formula:

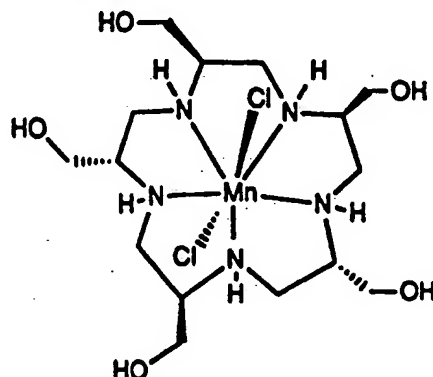


30

35

-15-

Another group of currently preferred compounds are those in which at least two of R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉ and R'₉ are alkyl or alkyl substituted with -OR₁₅ or -NR₁₅R₁₆ wherein R₁₅ and R₁₆ are independently hydrogen or alkyl. The number of "R" groups which are alkyl or substituted alkyl is preferably at least 3, and more preferably at least 5. An example of such a compound is represented by the formula:



A first embodiment of the invention relates to the above metal complexes wherein at least one of R₁₀, R₁₁, R₁₂, R₁₃ and R₁₄ is other than hydrogen.

A second embodiment of the invention relates to a method of magnetic resonance imaging comprising (a) administering to a human or non-human animal subject a contrast medium comprising a physiologically compatible complex of the invention and a nontoxic pharmaceutically acceptable carrier, adjuvant or vehicle; and (b) generating a magnetic resonance image of at least a part of the human or non-human animal subject.

A third embodiment of the invention relates to a method of diagnostic imaging comprising (a) administering to a human or non-human animal subject a diagnostic agent comprising a physiologically compatible complex of the present invention and a nontoxic, pharmaceutically acceptable carrier, adjuvant or

-16-

vehicle, and (b) generating an X-ray, ultrasound or scintigraphic image of at least a part of the human or non-human animal subject.

A fourth embodiment of the invention relates to a
5 method of radiotherapy practiced on a human or non-human animal subject comprising administering to the human or non-human animal subject a radioactive agent comprising a physiologically compatible complex of the present invention wherein M is a radioactive metal, and a
10 nontoxic, pharmaceutically acceptable carrier, adjuvant or vehicle.

The macrocyclic ligand used herein as a comparison to the complexes of the present invention wherein all R's are H can be prepared according to the general
15 synthetic scheme A set forth below utilizing methods known in the art for preparation of certain intermediates and certain ligands. See, for example, Richman et al., *J. Am. Chem. Soc.*, 96, 2268 (1974); Atkins et al. *Org. Synth.*, 58, 86 (1978); and EP 287
20 465. Thus a triazaalkane is tosylated in a suitable solvent system to produce the corresponding tris(N-tosyl) derivative. Such derivative is then treated with a suitable base to produce the corresponding disulfonamide anion. The disulfonamide anion is then
25 reacted with a di-O-tosylated di-N-tosylated diazaalkane diol to produce the corresponding pentatosylpentaazacycloalkane. The tosyl groups are then removed and the resulting compound is reacted with a manganese(II) compound under essentially anhydrous and
30 anaerobic conditions to form the corresponding manganese(II) pentaazacycloalkane complex.

The macrocyclic ligands useful in the complexes of the present invention, wherein R_1 , R'_1 , R_3 , R'_3 , R_5 , R'_5 , R_7 , R'_7 , R_9 and R'_9 can be H or any functionality as
35 previously described, can be prepared according to the general peptide method shown in Scheme B set forth

-17-

below. The procedure for preparing the cyclic peptide precursors from the corresponding linear peptides are the same or significant modifications of methods known in the art. See, for example, Veber, D.F. et al., J. Org. Chem., 44, 3101 (1979). The general method outlined in Scheme B below is an example utilizing the sequential solution-phase preparation of the functionalized linear pentapeptide from N-terminus to C-terminus. Alternatively, the reaction sequence to prepare the linear pentapeptide can be carried out by solid-phase preparation utilizing methods known in the art. The reaction sequence could be conducted from C-terminus to N-terminus and by convergent approaches such as the coupling of di- and tri-peptides as needed. Thus a Boc-protected amino acid is coupled with an amino acid ester using standard peptide coupling reagents. The new Boc-dipeptide ester is then saponified to the free acid which is coupled again to another amino acid ester. The resulting Boc-tri-peptide ester is again saponified and this method is continued until the Boc-protected pentapeptide free acid has been prepared. The Boc protecting group is removed under standard conditions and the resulting pentapeptide or salt thereof is converted to the cyclic pentapeptide. The cyclic pentapeptide is then reduced to the pentaazacyclopentadecane with lithium aluminum hydride or borane. The final ligand is then reacted with a manganese(II) compound under essentially anaerobic conditions to form the corresponding manganese(II) pentaazacyclopentadecane complex.

Scheme B was utilized for the synthesis of the complexes of Examples 3 and 5.

The R groups in the macrocycles produced by the cyclic peptide route, i.e., R_1 , R'_1 , R_3 , R'_3 , R_5 , R'_5 , R_7 , R'_7 , R_9 and R'_9 , could be derived from the D or L forms of the amino acids Alanine, Aspartic acid,

-18-

Arginine, Asparagine, Cysteine, Glycine, Glutamic acid, Glutamine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Proline, Phenylalanine, Serine, Tryptophan, Threonine, Tyrosine, Valine and /or the R groups of
5 unnatural α -amino acids such as alkyl, ethyl, butyl, tert-butyl, cycloalkyl, phenyl, alkenyl, allyl, alkynyl, aryl, heteroaryl, polycycloalkyl, polycycloaryl, polycycloheteroaryl, imines, aminoalkyl, hydroxyalkyl, hydroxyl, phenol, amine oxides, thioalkyl,
10 carboalkoxyalkyl, carboxylic acids and their derivatives, keto, ether, aldehyde, amine, nitrile, halo, thiol, sulfoxide, sulfone, sulfonic acid, sulfide, disulfide, phosphonic acid, phosphinic acid, phosphine oxides, sulfonamides, amides, amino acids, peptides,
15 proteins, carbohydrates, nucleic acids, fatty acids, lipids, nitro, hydroxylamines, hydroxamic acids, thiocarbonyls, borates, boranes, boraza, silyl, siloxy, silaza, and combinations thereof.

The macrocyclic ligands useful in the complexes
20 of the present invention can also be prepared by the diacid dichloride route shown in Scheme C set forth below. Thus, a triazaalkane is tosylated in a suitable solvent system to produce the corresponding tris(N-tosyl) derivative. Such a derivative is treated with a
25 suitable base to produce the corresponding disulfonamide anion. The disulfonamide anion is dialkylated with a suitable electrophile to produce a derivative of a dicarboxylic acid. This derivative of a dicarboxylic acid is treated to produce the dicarboxylic acid, which
30 is then treated with a suitable reagent to form the diacid dichloride. The desired vicinal diamine is obtained in any of several ways. One way which is useful is the preparation from an aldehyde by reaction with cyanide in the presence of ammonium chloride
35 followed by treatment with acid to produce the alpha ammonium nitrile. The latter compound is reduced in the

-19-

presence of acid and then treated with a suitable base to produce the vicinal diamine. Condensation of the diacid dichloride with the vicinal diamine in the presence of a suitable base forms the tris(tosyl)diamide
5 macrocycle. The tosyl groups are removed and the amides are reduced and the resulting compound is reacted with a manganese (II) compound under essentially anhydrous and anaerobic conditions to form the corresponding substituted pentaazacycloalkane manganese (II) complex.

10 Scheme C was utilized for the synthesis of the complexes of Example 2.

The vicinal diamines have been prepared by the route shown (known as the Strecker synthesis) and vicinal diamines were purchased when commercially
15 available. Any method of vicinal diamine preparation could be used.

The macrocyclic ligands useful in the complexes of the present invention can also be prepared by the bis(haloacetamide) route shown in Scheme D set forth
20 below. Thus a triazaalkane is tosylated in a suitable solvent system to produce the corresponding tris(N-tosyl) derivative. Such a derivative is treated with a suitable base to produce the corresponding disulfonamide anion. A bis(haloacetamide), e.g., a
25 bis(chloroacetamide), of a vicinal diamine is prepared by reaction of the diamine with an excess of haloacetyl halide, e.g., chloroacetyl chloride, in the presence of a base. The disulfonamide anion of the tris(N-tosyl) triazaalkane is then reacted with the
30 bis(chloroacetamide) of the diamine to produce the substituted tris(N-tosyl)diamide macrocycle. The tosyl groups are removed and the amides are reduced and the resulting compound is reacted with a manganese (II) compound under essentially anhydrous and anaerobic
35 conditions to form the corresponding substituted pentaazacycloalkane manganese (II) complex.

-20-

Scheme D is an alternative synthetic route to the complex of Example 2.

The macrocyclic ligands useful in the complexes of the present invention, wherein R_1 , R'_1 , R_2 , R'_2 are part of a *cis*- or *trans*- cycloalkyl ring system and R_5 , R'_5 , R_7 , R'_7 and R_9 , R'_9 can be H or any functionality previously described, can be prepared according to the pseudo-peptide method shown in Scheme E set forth below. A *cis*-1,2-Diaminocycloalkane or a *trans*-(*R,R*)-1,2-diaminocycloalkane or *trans*-(*S,S*)-1,2-diaminocycloalkane can be used in this method in combination with any amino acids. This allows the relative stereochemistry of the cycloalkane fused ring and substituent, R_5 , R'_5 , R_7 , R'_7 , R_9 , R'_9 , functionality and stereochemistry to be defined in any manner. As an example *trans*-(*R,R*)-1,2-diaminocyclohexane was monotosylated and reacted with Boc anhydride to afford the differentiated N-Boc, N-tosyl derivative. The sulfonamide was alkylated with methyl bromoacetate using sodium hydride as the base and saponified to the free acid. The cyclohexanediamine containing N-tosylglycine serves as a dipeptide surrogate in standard solution-phase peptide synthesis. Thus, coupling with a functionalized amino acid ester affords the corresponding pseudo-tripeptide. Two sequential TFA cleavage-couplings affords the pseudo-pentapeptide which can be N- and C-terminus deprotected in one step using HCl/AcOH. DPPA mediated cyclization followed by $LiAlH_4$ or Borane reduction affords the corresponding macrocyclic ligand. This ligand system is reacted with a manganese (II) compound, such as manganese (II) chloride under essentially anaerobic conditions to form the corresponding functionalized manganese (II) pentaazacycloalkane complex.

The macrocyclic ligands useful in the complexes of the present invention, wherein R_1 , R'_1 , R_2 , R'_2 and R_5 , R'_5 , R_6 , R'_6 , are part of a *cis*- or *trans*- cycloalkyl

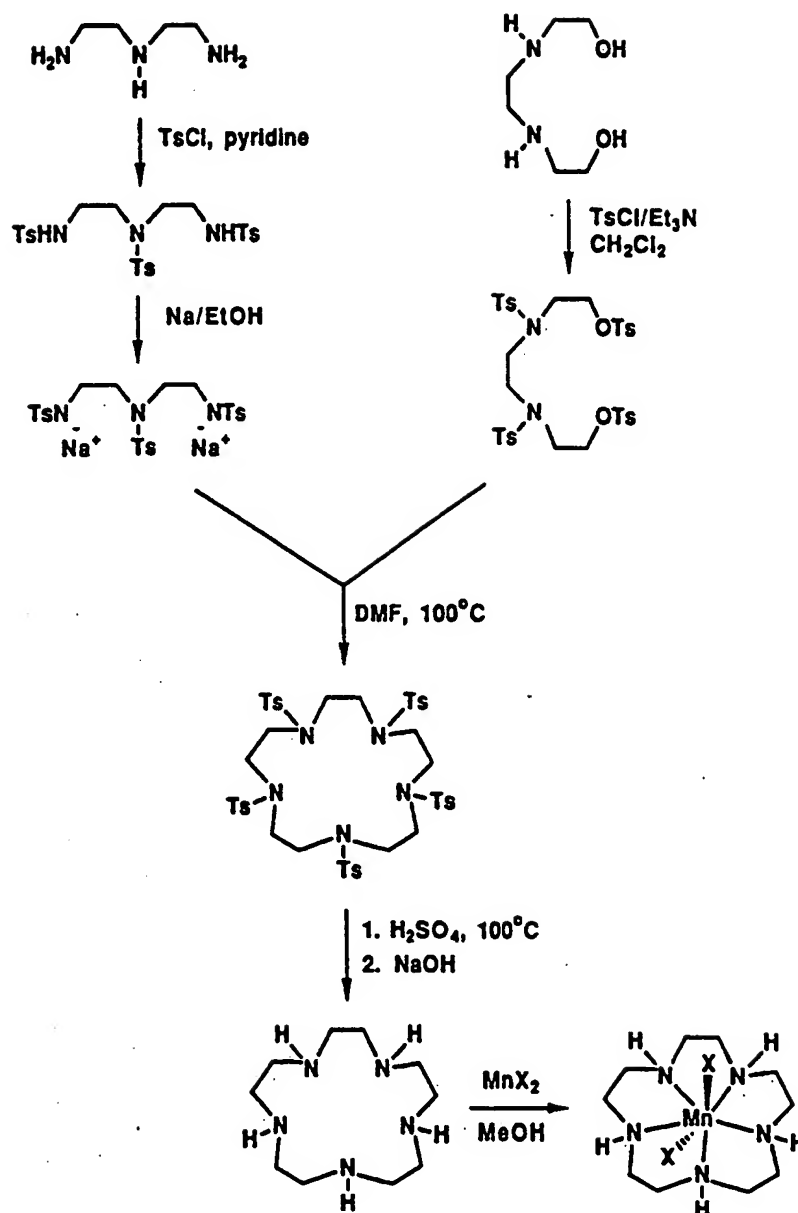
-21-

ring system and R_9 , R'_9 can be H or any functionality previously described, can be prepared according to the iterative pseudo-peptide method shown in Scheme F set forth below. A *cis*-1,2-Diaminocycloalkane or a *trans*-(R,R)-1,2-diaminocycloalkane or *trans*-(S,S)-1,2-diaminocycloalkane can be used in any combination with each other using this method and in combination with any amino acids. This allows the relative stereochemistry of both cycloalkane fused rings and substituent, R_9 , R'_9 , functionality and stereochemistry to be defined in any manner. Thus, the (S,S)-1,2-diaminocyclohexyl-N-tosylglycine dipeptide surrogate, prepared from (S,S)-1,2-diaminocyclohexane exactly as in Scheme E in the case of (R,R)-1,2-diaminocyclohexane, can be coupled with a functionalized amino acid ester to afford the corresponding pseudo-tripeptide. TFA cleavage affords the pseudo-tripeptide TFA salt which is coupled with (R,R)-diaminocyclohexyl-N-tosylglycine. Saponification and TFA cleavage affords the bis-cyclohexano containing pseudo-pentapeptide. DPPA mediated cyclization followed by $LiAlH_4$ or Borane reduction affords the corresponding bis-cyclohexano-fused macrocyclic ligand. This ligand system is reacted with a manganese (II) compound, such as manganese (II) chloride under essentially anaerobic conditions to form the corresponding functionalized manganese (II) pentaazacycloalkane complex.

-22-

SCHEME A

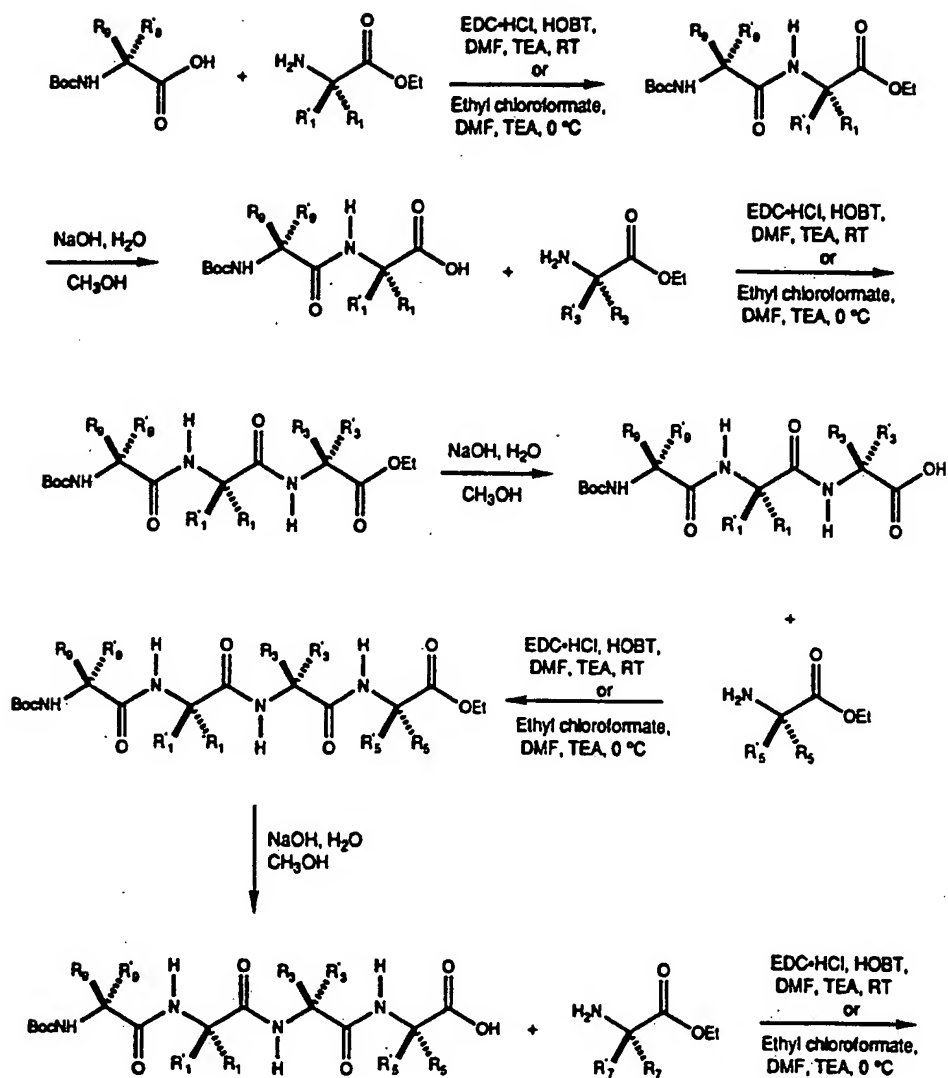
Scheme A



SUBSTITUTE SHEET (RULE 26)

-23-

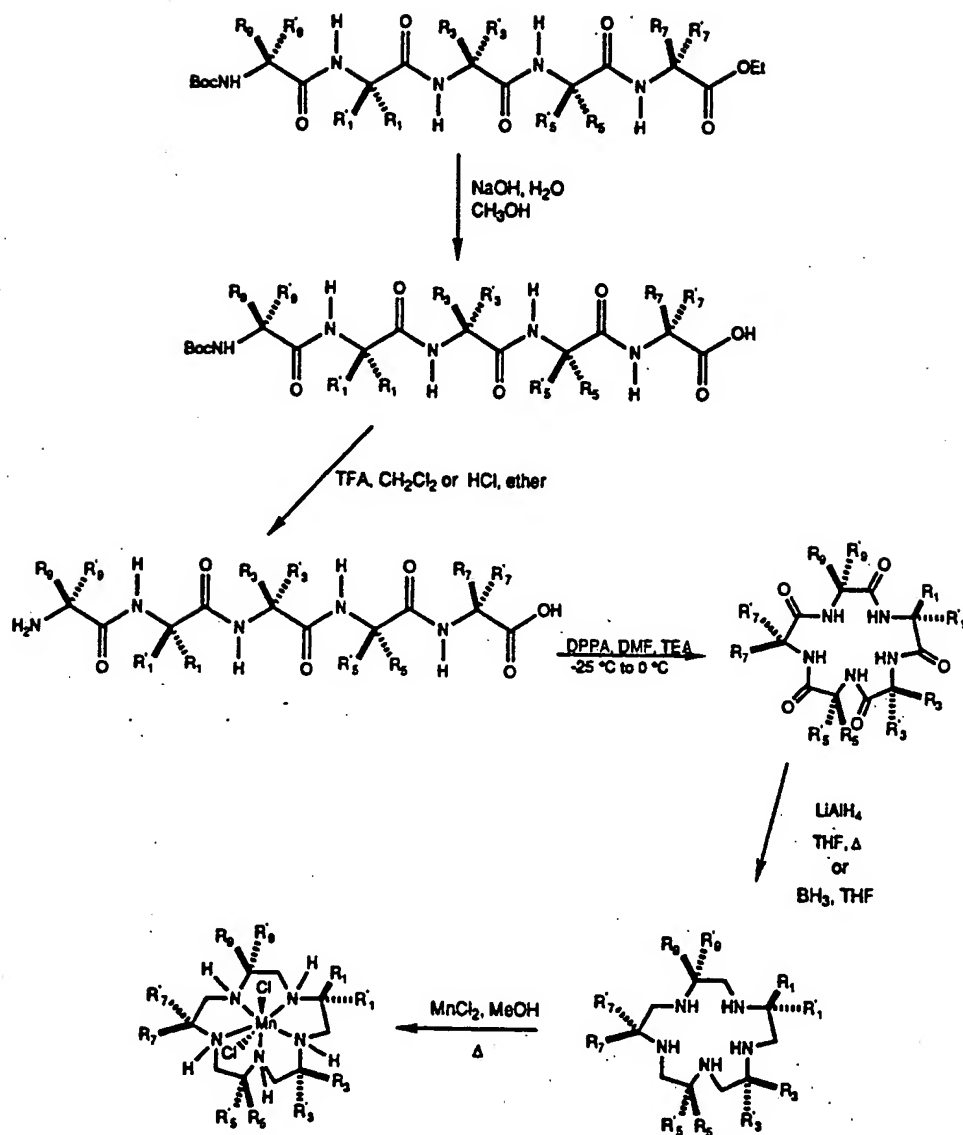
SCHEME B



SUBSTITUTE SHEET (RULE 26)

-24-

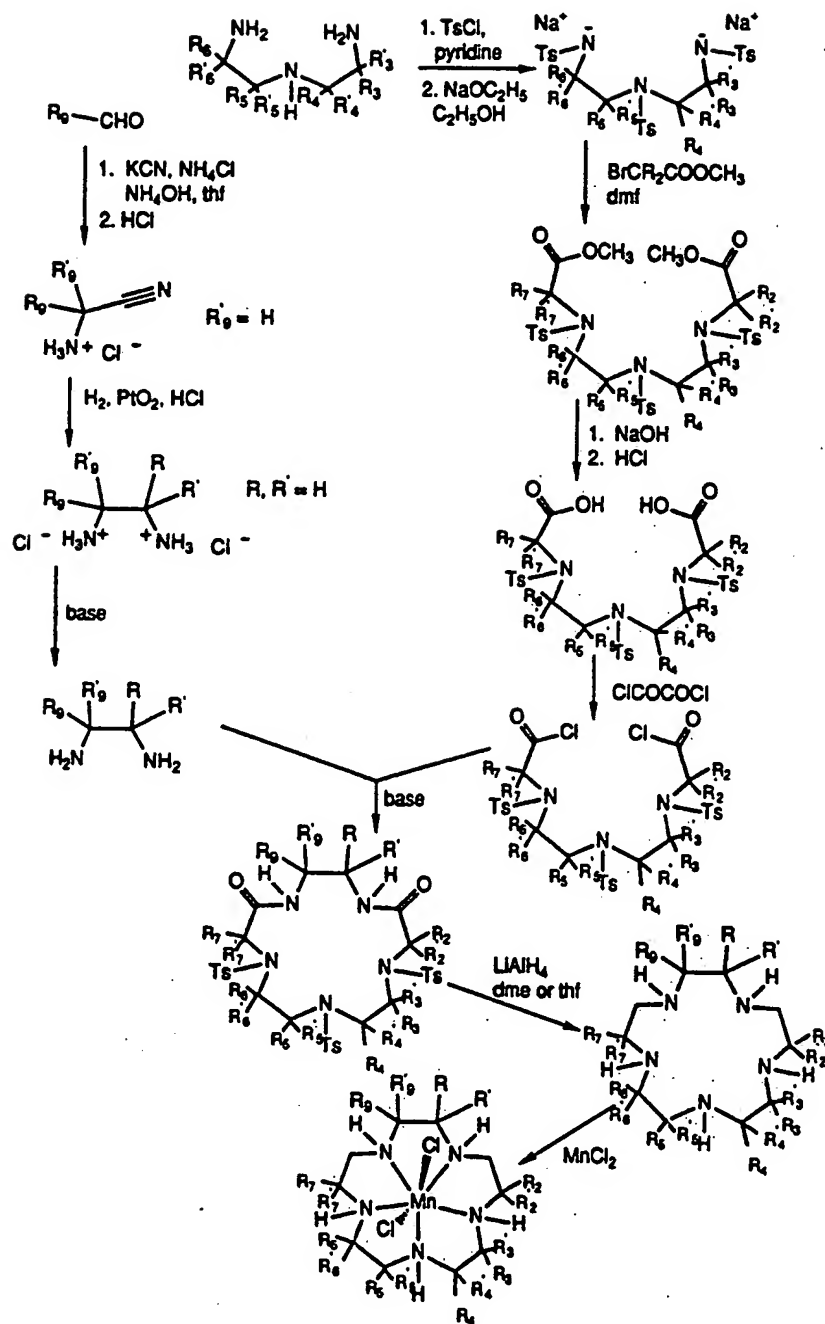
SCHEME B (Con't.)



SUBSTITUTE SHEET (RULE 26)

-25-

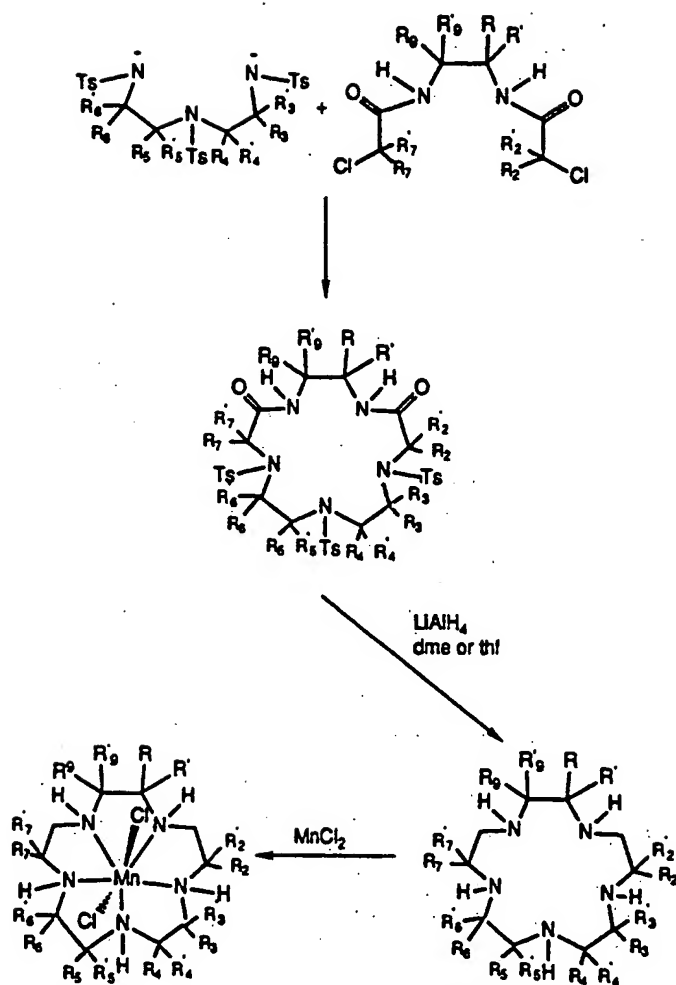
SCHEME C



SUBSTITUTE SHEET (RULE 26)

-26-

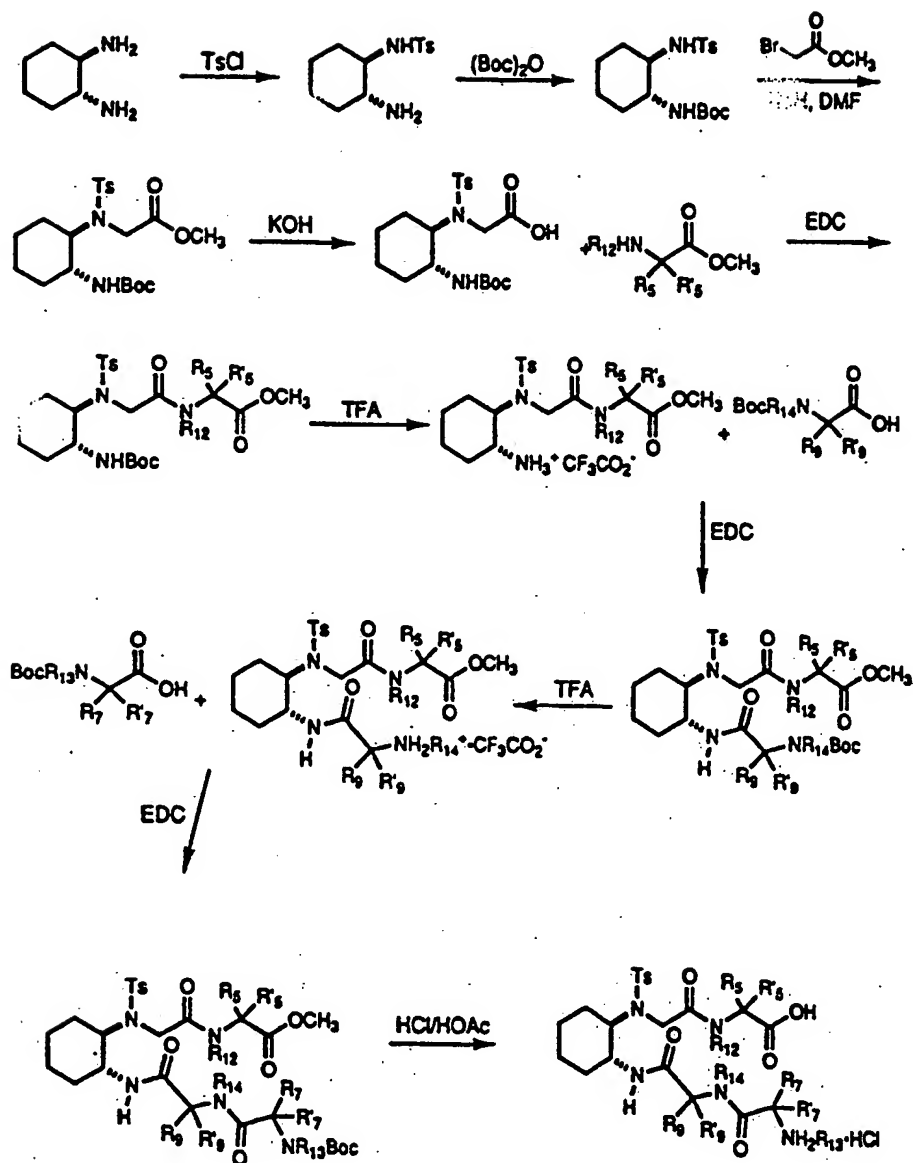
SCHEME D



SUBSTITUTE SHEET (RULE 26)

-27-

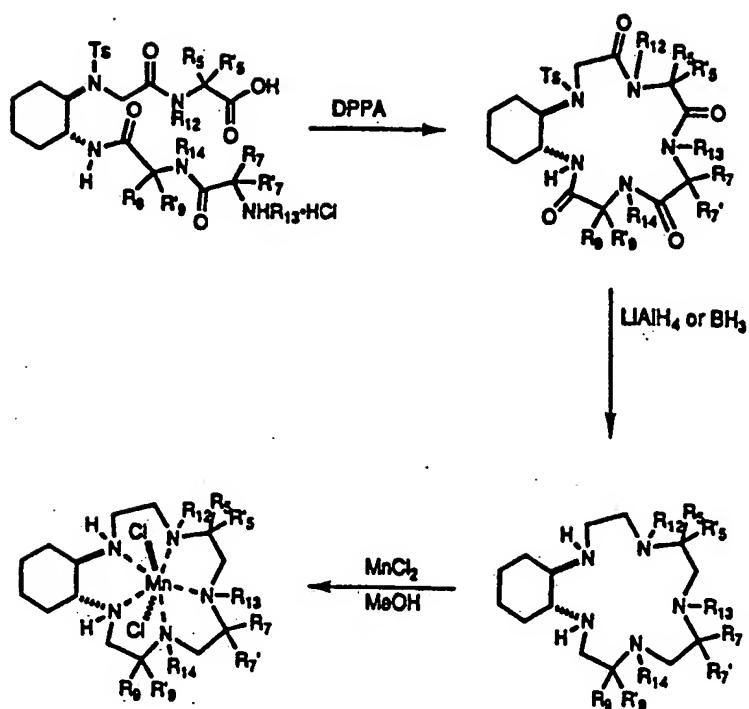
SCHEME E



SUBSTITUTE SHEET (RULE 26)

-28-

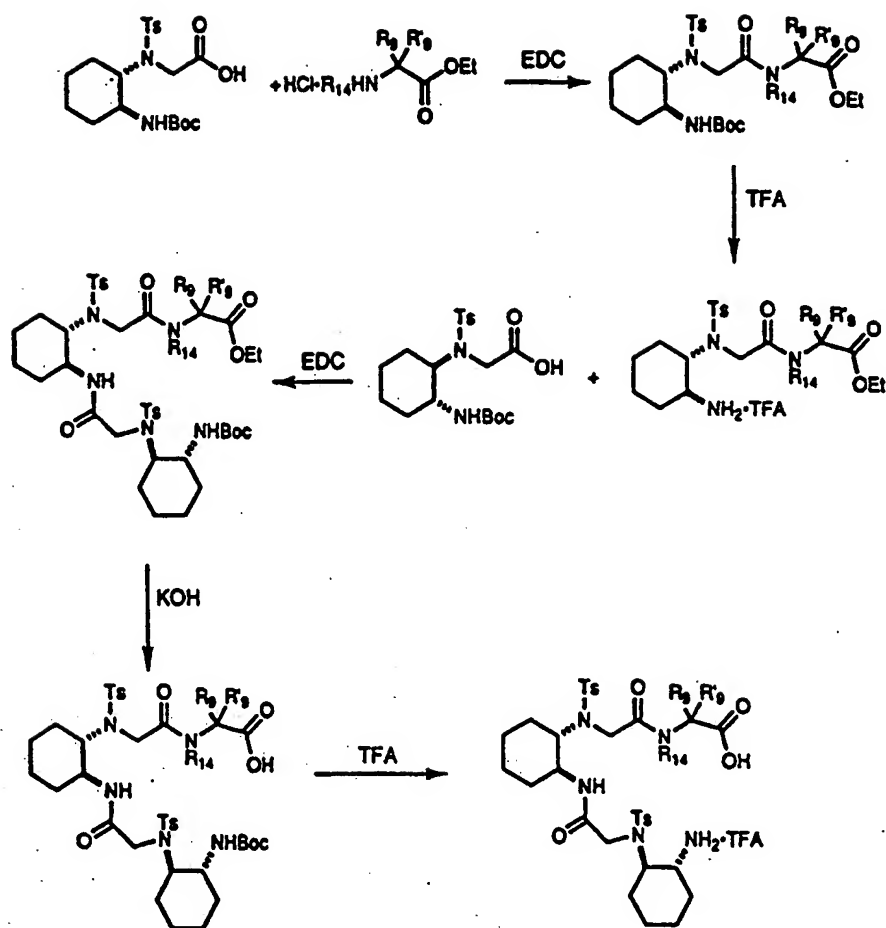
SCHEME E (Con't.)



SUBSTITUTE SHEET (RULE 28)

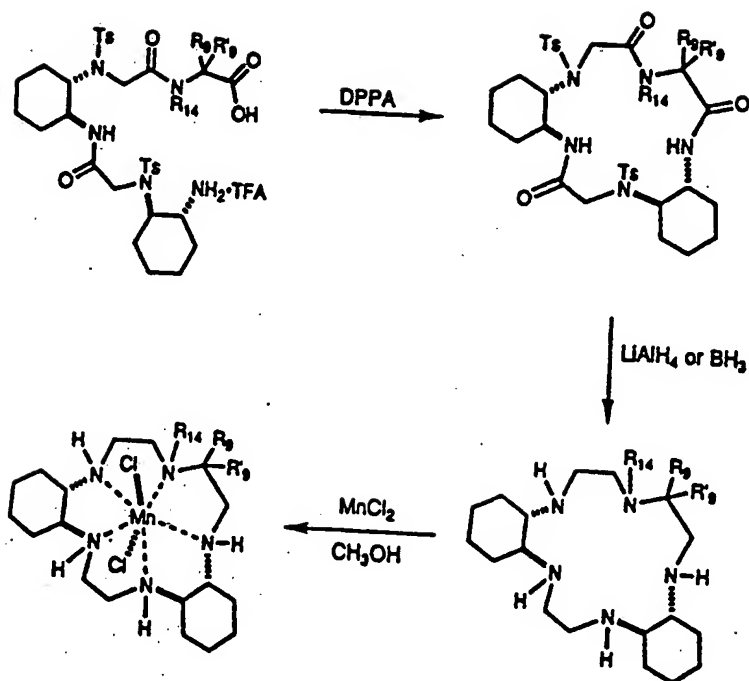
-29-

SCHEME F



-30-

SCHEME F (Con't.)



SUBSTITUTE SHEET (RULE 28)

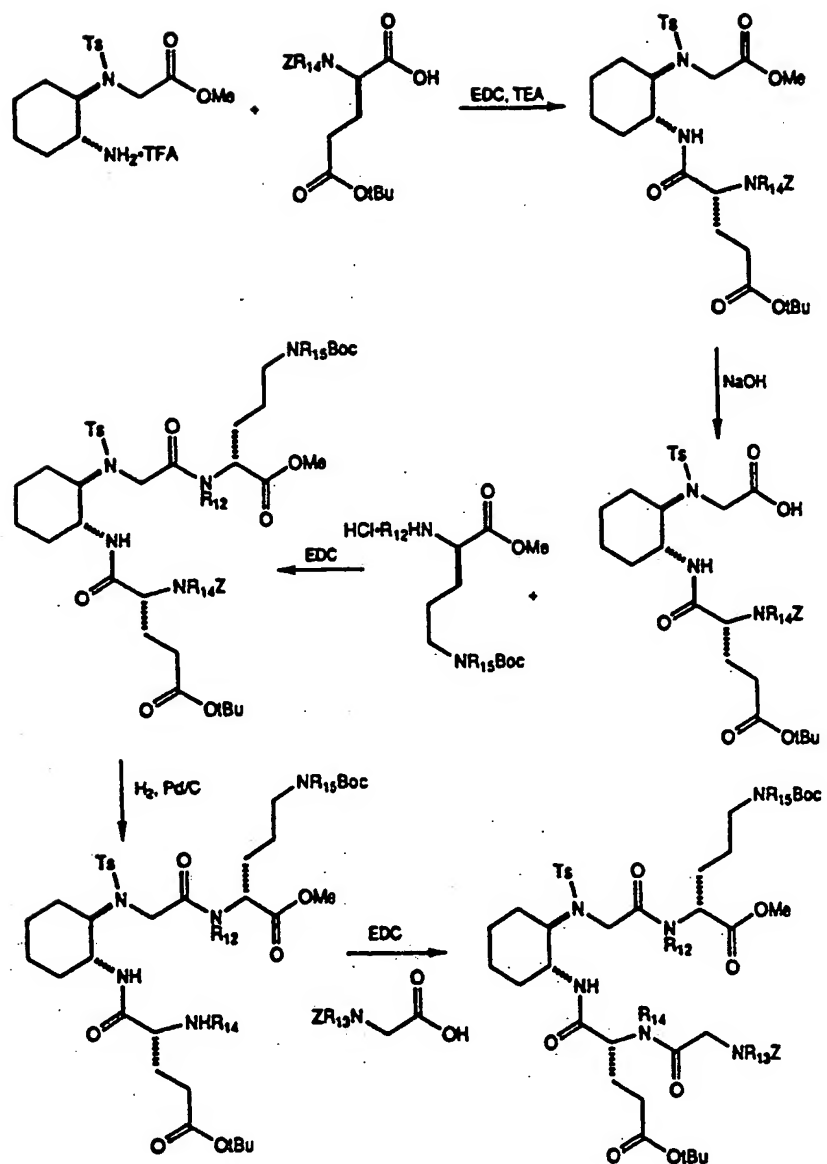
-31-

The macrocyclic ligands useful in the preparation of complexes of the present invention containing a strap can be prepared according to the following example schemes.

- 5 The macrocyclic ligands useful in the complexes of the present invention, wherein R_4 or R'_4 and R_8 or R'_8 are connected to one another through a "strap" or second ring system in a bicyclic sense, R_{12} , R_{13} and R_{14} can be H or any functionality previously described and R_{17} has
- 10 the same definition as R_{12} , R_{13} and R_{14} can be prepared according to the method set forth in Scheme G below. The TFA salt of trans-(R,R)-1,2-diaminocyclohexyl-N-tosylglycine dipeptide surrogate is coupled with
- 15 Z-Glu(OtBu)-OH using EDC in DMF. This tripeptide is then saponified and coupled with Orn(Boc)-OMe•HCl using EDC in DMF. The resulting tetrapeptide is then N-deprotected by hydrogenolysis of the Z group and coupled with Z-Gly again using EDC in DMF. Saponification and hydrogenolysis affords the
- 20 deprotected pseudopentapeptide which is cyclized with DPPA. The side chains are then deprotected in one step using TFA and a second "strap-cyclization" is effected again with DPPA as the coupling agent. Lithium aluminum hydride reduction affords the bicyclic ligand system
- 25 which is reacted with manganese (II) chloride under essentially anaerobic conditions to form the corresponding functionalized manganese (II) hexaazabicycloalkane complex.

-32-

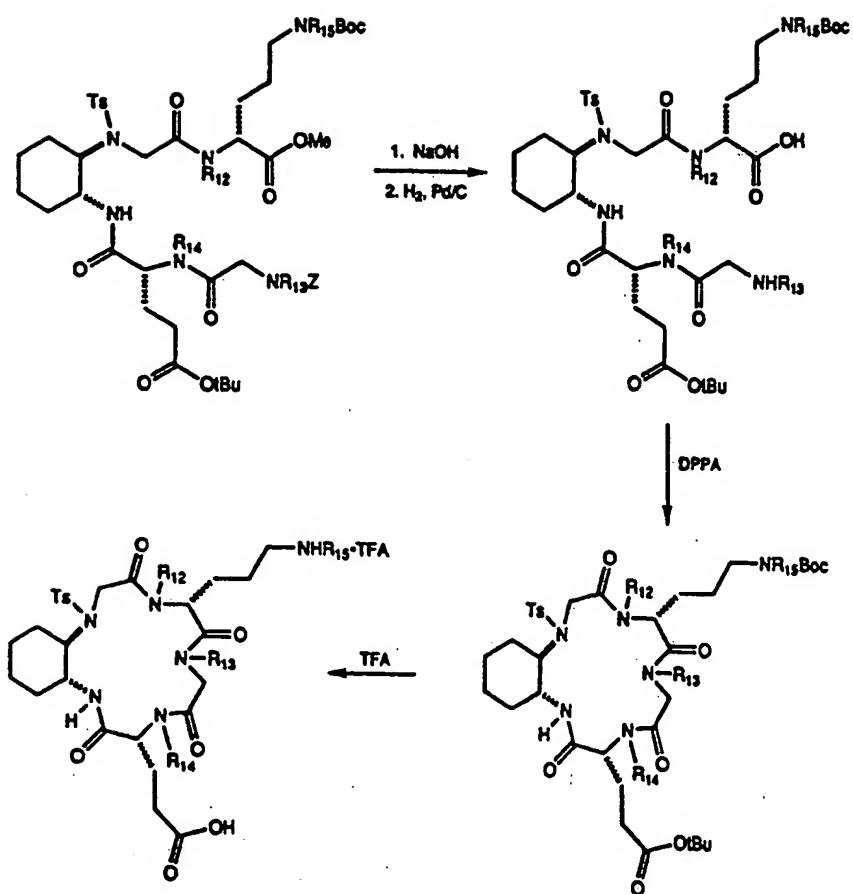
SCHEME G



SUBSTITUTE SHEET (RULE 26)

-33-

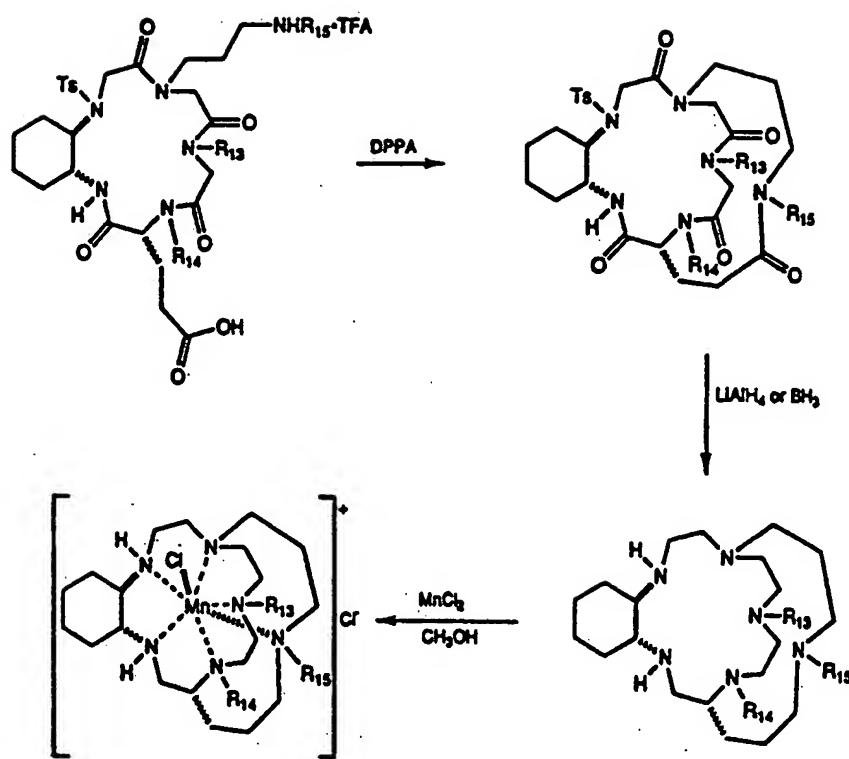
SCHEME G (Con't.)



SUBSTITUTE SHEET (RULE 28)

-34-

SCHEME G (Con't.)



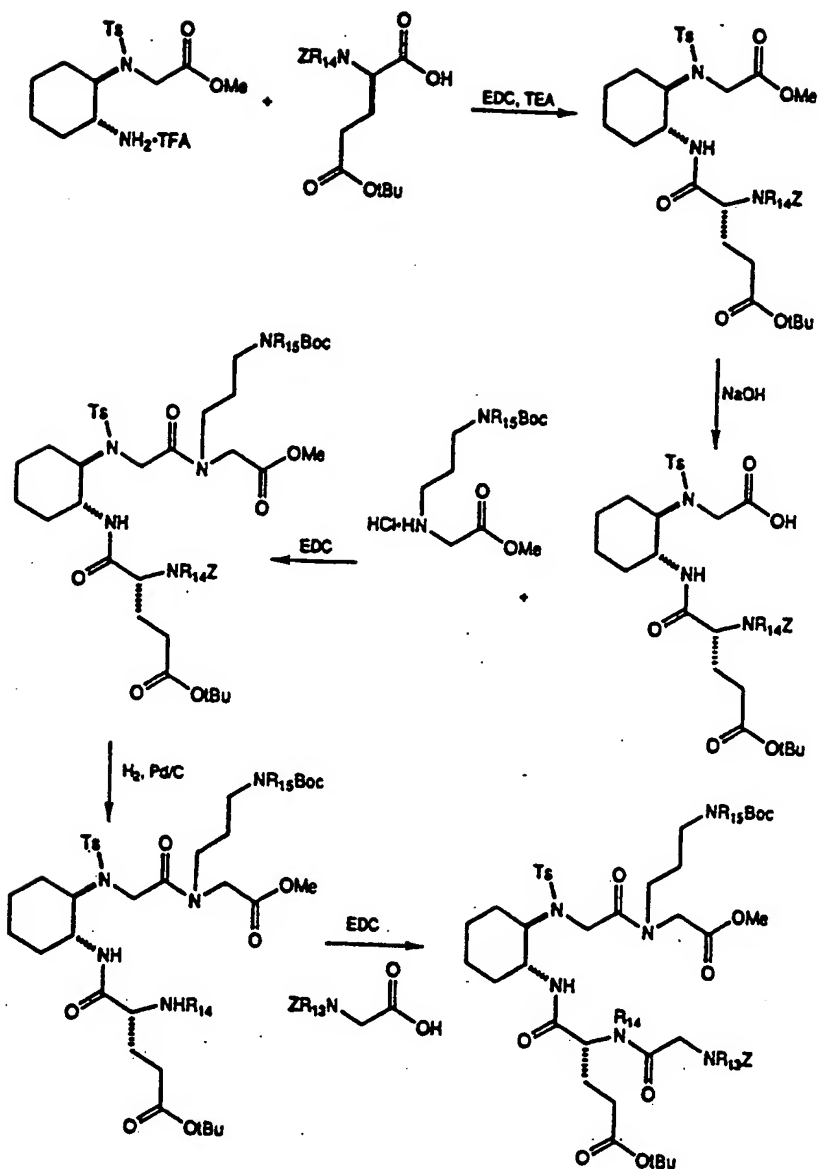
SUBSTITUTE SHEET (RULE 26)

-35-

The macrocyclic ligands useful in the complexes of the present invention, wherein nitrogen substituent R_{12} and R_8 or R'_8 are connected to one another through a "strap" or second ring system in a bicyclic sense and R_{13} , R_{14} and R_{17} can be H or any functionality previously described can be prepared according to the method set forth in Scheme H below. The TFA salt of trans-(R,R)-1,2-diaminocyclohexyl-N-tosylglycine dipeptide surrogate is coupled with Z-Glu(OtBu)-OH using EDC in DMF. This tripeptide is then saponified and coupled with N-[3-(Boc-amino)propyl]-Gly-OMe•HCl using EDC in DMF. The resulting tetrapeptide is then N-deprotected by hydrogenolysis of the Z group and coupled with Z-Gly again using EDC in DMF. Saponification and hydrogenolysis affords the deprotected pseudopentapeptide which is cyclized with DPPA. The side chains are then deprotected in one step using TFA and a second "strap-cyclization" is effected again with DPPA as the coupling agent. Lithium aluminum hydride reduction affords the bicyclic ligand system which is reacted with manganese (II) chloride under essentially anaerobic conditions to form the corresponding functionalized manganese (II) hexaazabicycloalkane complex.

-36-

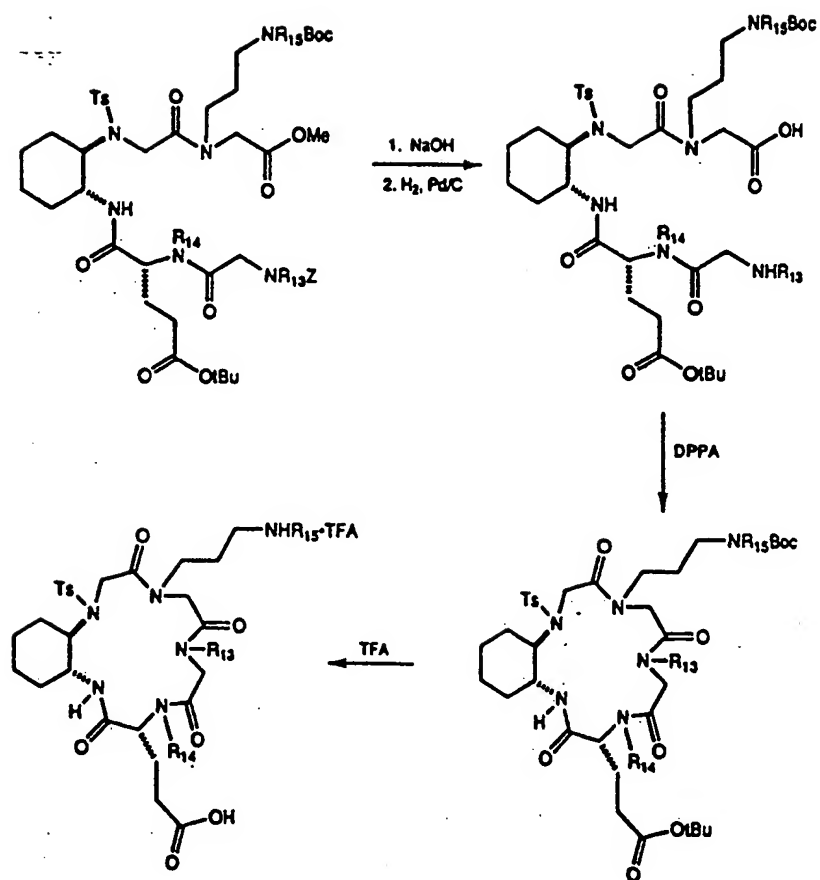
SCHEME H



SUBSTITUTE SHEET (RULE 28)

-37-

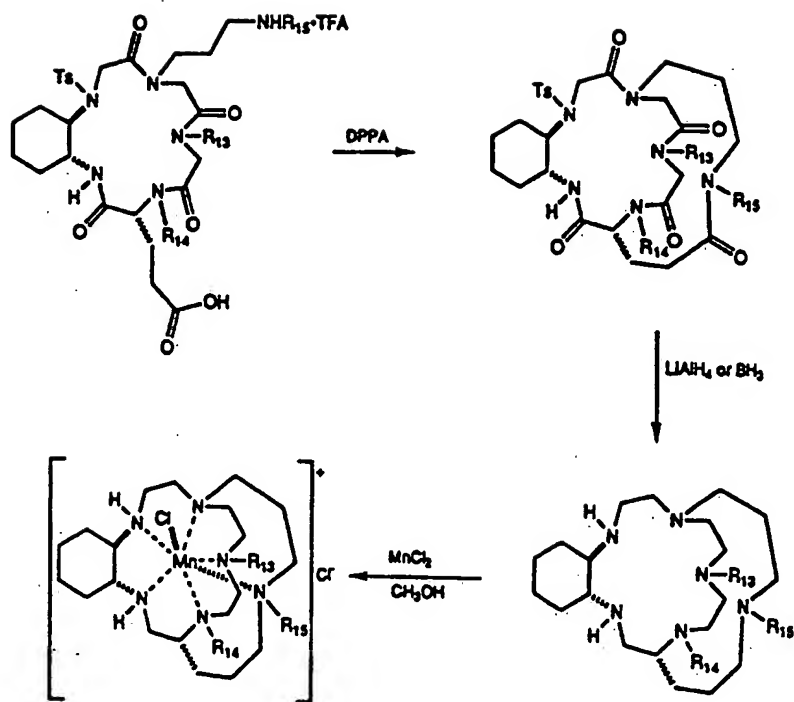
SCHEME H (Con't.)



SUBSTITUTE SHEET (RULE 26)

-38-

SCHEME H (Con't.)



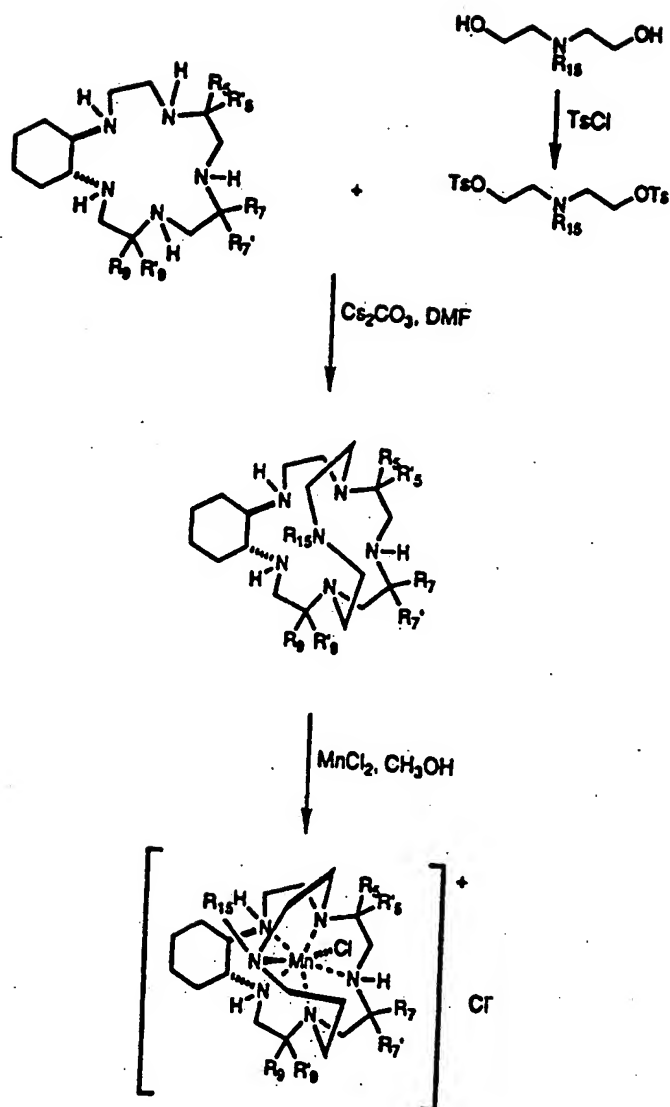
SUBSTITUTE SHEET (RULE 28)

-39-

The macrocyclic ligands useful in the complexes of the present invention, wherein nitrogen substituents R_{12} and R_{14} are connected to one another through a "strap" or
5 second ring system in a bicyclic sense and R_5 , R'_5 , R_7 , R'_7 , R_9 , R'_9 , and R_{17} can be H or any functionality previously described can be prepared according to the method set forth in Scheme I below. The cyclohexyl-containing pentaazamacrocyclic ligand, prepared
10 according to Scheme E is reacted with the ditosyl-sulfonamide of diethanolamine, which is prepared from diethanoamine and tosyl chloride. This "strapping" cyclization can be conducted using Cs_2CO_3 as the base in DMF solvent. This bicyclic ligand system is then
15 reacted with manganese (II) chloride under essentially anaerobic conditions to form the corresponding functionalized manganese (II) hexaazabicycloalkane complex.

-40-

SCHEME I



SUBSTITUTE SHEET (RULE 28)

-41-

The pentaazamacrocycles of the present invention can possess one or more asymmetric carbon atoms and are thus capable of existing in the form of optical isomers as well as in the form of racemic or nonracemic mixtures thereof. The optical isomers can be obtained by resolution of the racemic mixtures according to conventional processes, for example by formation of diastereoisomeric salts by treatment with an optically active acid. Examples of appropriate acids are tartaric, diacetyltartaric, dibenzoyltartaric, ditoluoyltartaric and camphorsulfonic acid and then separation of the mixture of diastereoisomers by crystallization followed by liberation of the optically active bases from these salts. A different process for separation of optical isomers involves the use of a chiral chromatography column optimally chosen to maximize the separation of the enantiomers. Still another available method involves synthesis of covalent diastereoisomeric molecules by reacting one or more secondary amine group(s) of the compounds of the invention with an optically pure acid in an activated form or an optically pure isocyanate. The synthesized diastereoisomers can be separated by conventional means such as chromatography, distillation, crystallization or sublimation, and then hydrolyzed to deliver the enantiomerically pure ligand. The optically active compounds of the invention can likewise be obtained by utilizing optically active starting materials, such as natural amino acids.

The methods of diagnostic analysis of the present invention involve administering the complexes, i.e. contrast enhancing agents, of the invention to a human or non-human animal subject or host, in an amount sufficient to effect the desired contrast (or shift) and then subjecting the host to diagnostic analysis. Preferably diagnostic analysis is NMR analysis;

-42-

including and especially preferred, NMR imaging analysis (or MRI). Further, the complexes of the present invention are useful in diagnostic analysis by X-ray image analysis, ultrasonic analysis or scintigraphic analysis. While described primarily as contrast enhancing agents, the complexes of the invention can act as NMR shift reagents and such use is contemplated by the methods herein.

The complexes of the invention used as contrast enhancing agents are administered in an amount sufficient to effect the desired contrast. For NMR, this amount is an NMR signal effecting amount of the complex, i.e. any amount of said complex that will alter the spin-lattice, spin-spin or spin-echo relaxation times of an NMR signal or for a shift reagent, selectively shift the spectral position of a resonance nucleus relative to other similar nuclei. This alteration is effected in a manner in order to enhance the signals received from the subject under analysis either by reducing the aforementioned relaxation times or by increasing them with respect to an area of the host or the host per se which has had the complex administered to it. In another embodiment, the NMR signal effecting amount of the complex is that amount which in addition to changing the relaxation times of the NMR signals in the host, will also change such relaxation times sufficiently so that sharper lines of definition or higher contrast is obtained between those parts of the host that have and have not been administered the complex.

The relaxation time T_1 (called the spin-lattice) measures the rate at which magnetic energy is transferred from the resonance nuclei to all other energetic degrees of freedom excluding other resonance nuclei. The relaxation time T_2 (spin-spin) measures the rate of magnetization transfer to other resonance nuclei.

-43-

Another parameter which can be measured is the density ρ of the protons in the medium. As a first approximation, it represents the quantity of free water contained in the sample.

5 The image by nuclear magnetic resonance represented the distribution of these parameters ρ , T_1 , T_2 or their combination. The contrast between a given tissue and the adjacent tissues increases as a function of the
10 tissues containing more or less water or mobile protons and differing relaxation times. It is also possible to modify the contrast by varying one or more of these parameters (experimentally echoes of spins aiding the function of T_2 , or reversal-recovery of the magnetization permitting the local measurement of T_1).
15 Experience has shown that it was of greater interest to modify the relaxation time to improve the contrast of the image which can be accomplished, for example, with the contrast enhancing agents provided herein. The density of the protons (in practice those of water and
20 lipids) varies little between individual organs and often less between normal and pathological tissues. However, the relaxation characteristics are dependent on a larger number of factors (microscopic dynamics of the molecules, chemical exchange, paramagnetic disturbances, etc.) which are much more variable.
25

A detailed discussion of NMR and theoretical considerations in selecting the appropriate parameters for diagnostic analysis, e.g. MRI, is rendered in U.S. Pat. No. 4,749,560 which is incorporated herein by
30 reference. X-ray image analysis, ultrasonic diagnosis, scintigraphic image analysis and radiotherapy utilizing the complexes of the invention are all conducted in accordance with well-established techniques known to those of ordinary skill in the art.
35

-44-

Moreover, the method of diagnostic analysis of the invention allows tissue or organ-specific diagnostic analysis to be achieved. For example, the contrast enhancing agents can exhibit organ and tissue
5 specificity, e.g. biodifferential distribution, such as in myocardial tissue when the complexes of the invention are lipophilic in nature.

The complexes of the invention may be administered to a host as a pharmaceutical composition in a contrast-
10 enhancing amount. The pharmaceutical compositions contain a contrast-enhancing dosage of the contrast agents according to the invention together with a nontoxic pharmaceutically acceptable carrier, adjuvant or vehicle. The compositions can be administered by
15 well-known routes including oral, intravenous (if soluble), intramuscular, intranasal, intradermal, subcutaneous, parenteral, enteral and the like. Depending on the route of administration, the pharmaceutical composition may require protective
20 coatings.

The pharmaceutical forms suitable for injectable use includes sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all
25 cases the ultimate solution form must be sterile and fluid. Typical carriers include a solvent or dispersion medium containing, for example, water, buffered aqueous solutions (i.e. biocompatible buffers), ethanol, polyol (glycerol, propylene glycol, polyethylene glycol, and
30 the like), suitable mixtures thereof, surfactants or vegetable oils. Sterilization can be accomplished by any art recognized technique, including but not limited to, addition of antibacterial or antifungal agents, for example, paraben, chlorobutanol, phenol, sorbic acid,
35 thimerosal, and the like. Further, isotonic agents,

-45-

such as sugars or sodium chloride may be incorporated in the subject compositions.

Production of sterile injectable solutions containing the subject contrast agent is accomplished by incorporating these agents in the required amount in the appropriate solvent with various ingredients enumerated above, as required, followed by sterilization, preferably filter sterilization. To obtain a sterile powder, the above solutions are vacuum-dried or freeze-dried as necessary.

Solid dosage forms for oral administration may include capsules, tablets, pills, powders, granules and gels. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluent, e.g. lubricating agents such as magnesium stearate. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluent commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

The contrast agents of the inventions are thus compounded for convenient and effective administration in pharmaceutically effective amounts with a suitable pharmaceutically acceptable carrier, adjuvant or vehicle in a dosage which effects contrast enhancement. These amounts are preferably about 1 μmol to 1 mol of the contrast agent per liter and/or administered in doses of about 0.001 to 5 mmol/kg body weight. Preferred

-46-

compositions provide effective dosages of contrast agents in the range of about 0.001-5 mmol/kg for NMR diagnostics, preferably about 0.005-0.5 mmol/kg; in the range of about 0.1-5 mmol/kg for X-ray diagnostics; and
5 in the range of about 0.1-5 mmol/kg for ultrasound diagnostics. For scintigraphic diagnostics, the dose of the contrast agent should generally be lower than for NMR diagnostics, e.g. MRI. For radiotherapy, conventional doses known to those of ordinary skill in
10 the art can be used.

As used herein, a pharmaceutically acceptable carrier, adjuvant or vehicle includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic agents, and the like. The
15 use of such media and agents are well known in the art.

Contemplated equivalents of the general formulas set forth above for the compounds and derivatives as well as the intermediates are compounds otherwise corresponding thereto and having the same general
20 properties such as tautomers of the compounds and such as wherein one or more of the various R groups are simple variations of the substituents as defined therein, e.g., wherein R is a higher alkyl group than that indicated, or where the tosyl groups are other
25 nitrogen or oxygen protecting groups or wherein the O-tosyl is a halide. Anions having a charge other than 1, e.g., carbonate, phosphate, and hydrogen phosphate, can be used instead of anions having a charge of 1, so long as they do not adversely affect the overall
30 activity of the complex. However, using anions having a charge other than 1 will result in a slight modification of the general formula for the complex set forth above. In addition, where a substituent is designated as, or can be, a hydrogen, the exact chemical nature of a
35 substituent which is other than hydrogen at that position, e.g., a hydrocarbyl radical or a halogen,

-47-

hydroxy, amino and the like functional group, is not critical so long as it does not adversely affect the overall activity and/or synthesis procedure.

The chemical reactions described above are

5 generally disclosed in terms of their broadest application to the preparation of the compounds of this invention. Occasionally, the reactions may not be applicable as described to each compound included within the disclosed scope. The compounds for which this

10 occurs will be readily recognized by those skilled in the art. In all such cases, either the reactions can be successfully performed by conventional modifications known to those skilled in the art, e.g., by appropriate protection of interfering groups, by changing to

15 alternative conventional reagents, by routine modification of reaction conditions, and the like, or other reactions disclosed herein or otherwise conventional, will be applicable to the preparation of the corresponding compounds of this invention. In all

20 preparative methods, all starting materials are known or readily preparable from known starting materials.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its

25 fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

30

EXAMPLES

All reagents were used as received without purification unless otherwise indicated. All NMR spectra were obtained on a Varian VXR-300 or VXR-400

35 nuclear magnetic resonance spectrometer. Qualitative and quantitative mass spectroscopy was run on a Finnigan

-48-

MAT90, a Finnigan 4500 and a VG40-250T using m-nitrobenzyl alcohol(NBA) or m-nitrobenzyl alcohol/LiCl (NBA+Li). Melting points (mp) are uncorrected.

The following abbreviations relating to amino acids
5 and their protective groups are in accordance with the recommendation by IUPAC-IUB Commission on Biochemical Nomenclature (Biochemistry, 11, 1726 (1972)) and common usage.

	Ala	L-Alanine
10	DAla	D-Alanine
	Gly	Glycine
	Ser	L-Serine
	DSer	D-Serine
	Ppg	Propargylglycine
15	Tyr	L-Tyrosine
	Bzl	Benzyl
	Boc	tert-Butoxycarbonyl
	Et	Ethyl
	TFA	Trifluoroacetate
20	DMF	Dimethylformamide
	HOBT•H ₂ O	1-Hydroxy-(1H)-benzotriazole monohydrate
	EDC•HCl	1-(3-Dimethylaminopropyl)-3- ethylcarbodiimide hydrochloride
25	TEA	Triethylamine
	DMSO	Dimethylsulfoxide
	THF	Tetrahydrofuran
	DPPA	Diphenylphosphoryl azide
	DMPU	Dimethylpropyleneurea
30	c	concentration, g/cc
	DME	1,2-Dimethoxyethane

The abbreviation Cyc represents 1,2-cyclohexanediamine (stereochemistry, i.e. R,R or S,S, is
35 indicated as such). This allows three letter code

-49-

peptide nomenclature to be used in pseudopeptides containing the 1,2-cyclohexane diamine "residue".

Example 1 (Comparative)

5 A. Synthesis of 1,4,7-Tris(p-toluenesulfonyl)-1,4,7-triazaheptane

This compound was synthesized following the procedure of Atkins, T. J.; Richman, J.E.; and
10 Oettle, W.F.; *Org. Synth.*, 58, 86-98 (1978). To a stirred solution of p-toluenesulfonyl chloride (618 g, 3.24 mole) in pyridine (1500 ml) at 0°C was added a solution of 1,4,7-triazaheptane (95.5 g, 0.926 mole) in pyridine (150 ml) under a dry argon atmosphere,
15 maintaining the temperature $\leq 50^\circ\text{C}$. The addition required 30 minutes. After the mixture was allowed to cool to room temperature slowly while stirring for 3 h, H_2O (2 l) was slowly added to the cooled (ice bath) mixture. The heavy white precipitate which formed was
20 filtered and washed thoroughly with H_2O . The pale yellow solid was dissolved in DMF (3 l) and 0.1 N HCl (4 l) was slowly added at 5°C. The slurry was filtered and the pale yellow solid was washed thoroughly with H_2O and dried in vacuo to give 486 g (93% yield) of the product:
25 mp $180-1^\circ\text{C}$; ^1H NMR(DMSO- d_6) δ 2.39 (s, 3 H), 2.40 (s, 6 H), 2.84 (m, 4 H), 3.04 (t, J=6.9 Hz, 4 H) 7.40 (d, J=8.1 Hz, 4 H), 7.59 (d, J=8.3 Hz, 2 H), 7.67 (m, 6 H).
B. Synthesis of 1,4,7-Tris(p-toluenesulfonyl)-1,4,7-triazaheptane-1,7-disodium Salt

30 This compound was synthesized following the procedure of Atkins, T.J.; Richman, J.E., and Oettle, W.F.; *Org. Synth.*, 58 86-98 (1978). To a mechanically stirred slurry of 1,4,7-tris(p-toluenesulfonyl)-1,4,7-triazaheptane prepared as in Example 1A (486 g, 0.859
35 mole) in ethanol (1150 ml) heated to reflux under a dry argon atmosphere was added a solution of sodium ethoxide

-50-

(prepared by dissolving sodium metal (39.5 g, 1.72 mole) in absolute ethanol (1.0 l)) as rapidly as possible. The clear brown solution which formed rapidly was allowed to cool to room temperature and ethyl ether (1.0 l) was added. The crystals were filtered under a dry argon blanket, washed with 3:1 ethanol:ethyl ether and ethyl ether. The crystals were then dried in vacuo to give 509 g (97% yield) of the product as a white powder: ^1H NMR ($\text{DMSO}-d_6$) δ 2.30 (s, 6 H), 2.36 (s, 3 H), 2.63 (t, J=8.7 Hz, 4 H), 2.89 (t, J=7.2 Hz, 4 H) 7.11 (d, J=8.1 Hz, 4 H), 7.28 (d, J=8.0 Hz, 2 H), 7.46 (m, 6 H).

C. Synthesis of 3,6-Bis(p-toluenesulfonyl)-3,6-diazaoctane-1,8-di-p-toluenesulfonate

To a stirred solution of p-toluenesulfonyl chloride (566 g, 2.97 mole) and triethylamine (300 g, 2.97 mole) in CH_2Cl_2 (2.0 l) at 0°C under a dry argon atmosphere was added 3,6-diazaoctane-1,8-diol (100 g, 0.675 mole) in portions, maintaining the temperature $<10^\circ\text{C}$. The addition required 30 minutes. The mixture was allowed to warm to room temperature while stirring an additional 18 h and was then poured onto ice (1000 g). The CH_2Cl_2 layer was separated, washed with 10% HCl, H_2O and saturated NaCl solution, and dried (MgSO_4). The solution was concentrated in vacuo to a volume of 1.5 l. Crystallization by the addition of hexane (4 l) gave 477 g (92% yield) of the product as colorless needles: mp $151-3^\circ\text{C}$; ^1H NMR (CDCl_3) δ 2.43 (s, 12 H), 3.29 (s, 4 H), 3.36 (t, J=5.2 Hz, 4 H), 4.14 (t, J=5.2 Hz, 4 H), 7.33 (d, J=7.8 Hz, 8 H), 7.71 (d, J=8.2 Hz, 4 H), 7.79 (d, J=8.3 Hz, 4 H).

D. Synthesis of 1,4,7,10,13-Penta(p-toluenesulfonyl)-1,4,7,10,13-pentaazacyclopentadecane

This compound was synthesized following the procedure of Richman, J.E., and Atkins, T.J., J. Am. Chem. Soc., 96, 2268-70 (1974). To a stirred solution of 1,4,7-tris(p-toluenesulfonyl)-1,4,7-triazaheptane-

-51-

1,7-disodium salt prepared as in Example 1B (146 g, 0.240 mole) in anhydrous DMF (2250 ml) was added dropwise over 3 h to a solution of 3,6-bis(p-toluenesulfonyl)-3,6-diazaoctane-1,8-di-p-toluenesulfonate prepared as in Example 1C (184 g, 0.240 mole) in anhydrous DMF (1020 ml) under a dry argon atmosphere, maintaining the temperature at 100°C. After stirring an additional 1 h at 100°C, the solution was concentrated in vacuo to a volume of 1.5 l. H₂O (500 ml) was slowly added at 80°C to crystallize the product. The resulting slurry was slowly cooled to 0°C and additional H₂O (1250 ml) added. The solid was filtered, washed thoroughly with H₂O and then 90% ethanol and dried in vacuo. The off-white solid was dissolved in CH₂Cl₂, insoluble impurities were removed by filtration and the filtrate was washed with H₂O and then dried (MgSO₄). The solvent was removed in vacuo to give a yellow solid which was purified by recrystallization from CH₂Cl₂-hexane to give 164 g (69% yield) of the product as a white crystalline solid: mp 290-3°C; ¹H NMR (CDCl₃) δ 2.44 (s, 15 H) 3.27 (s, 20 H), 7.32 (d, J=8.3 Hz, 10 H), 7.66 (d, J=8.3 Hz, 10 H).

E. Synthesis of 1,4,7,10,13-Pentaazacyclopentadecane

A mixture of 1,4,7,10,13-penta(p-toluenesulfonyl)-1,4,7,10,13-pentaazacyclopentadecane prepared as in Example 1D (168 g, 0.170 mole) and concentrated H₂SO₄ (500 ml) was heated at 100°C with stirring under a dry argon atmosphere for 70 h. To the resulting dark brown solution ethanol (500 ml) was added dropwise with stirring at 0°C followed by ethyl ether (3 l). The white solid was filtered and washed with ethyl ether. The solid was then dissolved in H₂O (500 ml) and the resulting solution washed with ethyl ether. Upon reducing the volume of the solution in vacuo to 200 ml, the pH was adjusted to 10-11 with 10 N NaOH and the solvent was removed in vacuo. Ethanol (500 ml) was then

-52-

added and removed in vacuo to dryness. The resulting tan oily solid was extracted with hot THF (2x500 ml) and filtered at room temperature. The filtrates were combined and the solvent removed in vacuo to give the crude product as a yellow crystalline solid which was then redissolved in CH_3CN and filtered to remove insoluble impurities. Recrystallization from cold (-20°C) CH_3CN gave 11.3 g (31% yield) of the product as colorless needles: mp $108-9^\circ\text{C}$; ^1H NMR (CDCl_3) δ 1.74 (br s, 5 H), 2.73 (s, 20 H); Exact mass ($\text{M}+\text{Li}$) $^+$: calcd, 222.2270; Found, 222.2269 ($\text{C}_{10}\text{H}_{25}\text{N}_5\text{Li}$).

F. Synthesis of [Manganese(II)dichloro(1,4,7,10,13-Pentaazacyclopentadecane)]

A solution of 1,4,7,10,13-pentaazacyclopentadecane prepared as in Example 1E (2.0 g, 9.3 mmole) and anhydrous manganese(II) chloride (1.2 g, 9.3 mmole) in anhydrous methanol (50 ml) was refluxed under a dry nitrogen atmosphere for 3 h. The solution was filtered and the solvent removed in vacuo. The resulting solid was recrystallized from ethanol-ethyl ether to give 2.79 g (88% yield) of the product as an off-white crystalline solid: FAB mass spectrum (NBA) m/z (relative intensity) 340 (M^+ , 2), 305/307 ($(\text{M}-\text{Cl})^+$ 100/45); Anal. Calcd. for $\text{C}_{10}\text{H}_{25}\text{Cl}_2\text{MnN}_5$: C, 35.17; H, 7.38; Cl, 20.76; N 20.60. Found: C, 34.95; H, 7.31; Cl, 20.49; N, 20.22.

Example 2

A. Synthesis of Dimethyl 3,6,9-Tris(p-toluenesulfonyl)-3,6,9-triazaundecanedioate

1,4,7-Tris(p-toluenesulfonyl)-1,4,7-triazaheptane-1,7-disodium salt prepared as in Example 1B (30 g, 49.2 mmol) was dissolved in dry N,N -dimethylformamide (180 ml) under argon. After cooling to 0°C in an ice bath, methyl chloroacetate (15.40 g, 141.9 mmol) was added dropwise over a 10 min period. The reaction mixture

-53-

became cloudy at the end of the addition, and was allowed to stir overnight while the ice bath warmed to room temperature. The solvent was evaporated under reduced pressure to give a brown oil which was dissolved in ethyl acetate (450 ml) giving a milky solution. This solution was washed twice with water (500 ml, then 300 ml). The combined water layers were back extracted with ethyl acetate (300 ml). The combined ethyl acetate layers were washed twice with saturated sodium chloride solution (200 ml), filtered, and evaporated to dryness. This residue was dissolved in dichloromethane (200 ml) and evaporated to dryness, and placed on the vacuum line. After recrystallization from chloroform-methanol, and washing with methanol and ether, an off-white solid was obtained weighing 27.46 g. An additional quantity of a slightly darker solid (4.7 g) was recovered from the filtrate after removing the solvent and recrystallizing as before. Total yield was 32.2 g (93% yield): mp 141-2° C; ¹H NMR (CDCl₃) δ 2.42 and 2.44 (2 s, 9 H), 3.41 (br s, 8 H), 3.60 (s, 6 H), 4.07 (s, 4 H), 7.26 - 7.35 (m, 6 H), 7.63 - 7.74 (m, 6 H).

B. Synthesis of 3,6,9-Tris(p-toluenesulfonyl)-3,6,9-triazaundecanedioic Acid

Dimethyl 3,6,9-tris(p-toluenesulfonyl)-3,6,9-triazaundecanedioate prepared as in Example 2A (16 g, 22.5 mmol) was slurried in tetrahydrofuran (100 ml). Sodium hydroxide (2 N, 160 ml) was added dropwise over a 1 h period. After 72 h, the solvent was evaporated under reduced pressure, and hydrochloric acid (1 N) was added to lower the pH to 4. This aqueous phase was extracted several times with ethyl acetate. The combined ethyl acetate layers were washed twice with brine, dried (MgSO₄), filtered, and evaporated to give a white solid, 14.22 g (93% yield): mp 177-80° C; ¹H NMR (DMSO-d₆) δ 2.38 and 2.40 (2 s, 9 H), 3.10 (m, 4 H), 3.29 (m, 4 H), 3.73 (s, 4 H), 7.37 and 7.41 (2 d, J =

-54-

7.9, 8.2 Hz, 6 H), 7.61 and 7.66 (2 d, $J = 8.2$, 8.0 Hz, 6 H).

C. Synthesis of 3,6,9-Tris(p-toluenesulfonyl)-3,6,9-triazaundecanedioyl Dichloride

5 3,6,9-Tris(p-toluenesulfonyl)-3,6,9-triazaundecanedioic acid prepared as in Example 2B (40.5 g, 59.4 mmol) was placed in a round bottom flask under argon, and oxalyl chloride (400 g, 3.15 moles) was added. This mixture, initially cloudy, became clear
10 after a few hours, and was stirred overnight at room temperature. At the end of this time it was heated to 40° C for 30 min. Oxalyl chloride was removed on the rotary evaporator. Dichloromethane (50 to 60 ml) was added to dissolve the resulting solid, and was removed
15 on the rotary evaporator. This process was repeated twice, giving 40.5 g (99% yield) of a white solid: mp 136-7° C; ^1H NMR (CDCl_3) δ 2.43 and 2.46 (2 s, 9 H), 3.30 - 3.38 (m, 4 H), 3.40 - 3.48 (m, 4 H), 4.56 (s, 4 H), 7.30 - 7.40 (m, 6 H), 7.71 (d, $J = 8.2$ Hz, 6 H).

20

D. Synthesis of trans-5,6-Cyclohexano-1,10,13-tris(p-toluenesulfonyl)-1,4,7,10,13-pentaazacyclopentadecan-3,8-dione

25 Dry dichloromethane (150 ml) was placed in a one liter four necked round bottom flask under argon equipped with two dropping funnels. 3,6,9-Tris(p-toluenesulfonyl)-3,6,9-triazaundecanedioyl dichloride prepared as in Example 2C (5.07 g, 7.05 mmol) was dissolved in dry dichloromethane (150 ml) and added to
30 one of the dropping funnels. trans-1,2-Diaminocyclohexane (0.805 g, 7.05 mmol) and triethylamine (1.96 ml, 14.1 mmol) were dissolved in dry dichloromethane (150 ml) and added to the other dropping funnel. After cooling the dichloromethane containing
35 flask in an ice bath to an internal temperature of 0 to 5° C, the contents of the dropping funnels were added

-55-

simultaneously to the stirred solution over 2.25 h. A white precipitate was evident before the addition was finished. At the end of this time, the ice bath was removed and the reaction mixture was stirred overnight at room temperature. The reaction mixture was filtered and the white precipitate was identified as pure product. The filtrate was washed twice with water (100 ml), once with saturated sodium chloride solution (100 ml), dried (MgSO_4), filtered, and the solvent was removed under reduced pressure. Recrystallization from dichloromethane-hexane provided additional product, along with the initial precipitate for a total of 2.85 g (53% yield); mp 254-5 °C; ^1H NMR ($\text{DMSO}-d_6$) δ 1.15 (br s, 4 H), 1.52 - 1.75 (m, 4 H), 2.42 and 2.43 (2 s, 9 H), 3.04 (m, 8 H), 3.51 (d + m, $J = 16.5$ Hz, 4 H), 4.01 (d, $J = 16.5$ Hz, 2 H), 7.35 - 7.53 (m, 8 H), 7.71 (d, $J = 8.3$ Hz, 4 H), 7.80 (br d, $J = 10.5$ Hz, 2 H).

E. Synthesis of trans-1,2-

20 Bis(chloroacetamido)cyclohexane

A 12 liter three-neck flask equipped with a magnetic stirbar and two 1 liter dropping funnels was charged with 1,2-diaminocyclohexane (35.0 g, 0.310 mol) dissolved in chloroform (375 ml) and water (185 ml). The two dropping funnels were charged individually with chloroacetyl chloride (75 ml, 0.94 mol) in chloroform (440 ml) and potassium carbonate (120.5 g, 0.87 mol) in water (4 l), added in four portions during the addition. The flask was cooled in an ice salt bath and addition of the reagents was carried out over 2 h. The ice salt bath was removed, water (600 ml) was added, and the reaction mixture was stirred for 2.5 h. The mixture was separated and the water layer was extracted with chloroform several times. The combined chloroform layers were washed with water and then brine. The organic layer was dried (sodium sulfate) and

-56-

concentrated in vacuo to yield an off-white solid. The solid was washed with ether to yield 55.32 g (67% yield) of a white solid after drying in vacuo: mp 202-3 °C; ¹H NMR (CDCl₃) δ 1.27 - 1.50 (m, 4 H), 1.75 - 1.95 (m, 2 H), 2.03 - 2.20 (m, 2 H), 3.72 - 3.87 (m, 2 H), 4.05 (s, 4 H), 6.81 (br s, 2 H).

F. Synthesis of trans-5,6-Cyclohexano-1,10,13-tris(p-toluenesulfonyl)-1,4,7,10,13-pentaazacyclopentadecan-3,8-dione (alternate method)

A 5 liter three-neck flask equipped with a magnetic stir bar and 1 liter dropping funnel was dried and placed under a dry argon atmosphere. A solution of trans-1,2-bis(chloroacetamido)cyclohexane prepared as in Example 2E (6.68 g, 250 mmol) in anhydrous DMF (1.25 l) was added to a solution of 1,4,7-tris(p-toluenesulfonyl)-1,4,7-triazaheptane-1,7-disodium salt prepared as in Example 1B (15.2 g, 250 mmol) in anhydrous DMF (1.25 l) at room temperature over a period of 12 h. After stirring an additional 2 h, the solvent was removed in vacuo. The solid residue was triturated with chloroform (1 l) and filtered to yield a white solid. The solid was recrystallized from acetonitrile to give 7.22 g (38% yield) of fluffy white crystals: mp 254-5 °C; ¹H NMR (DMSO-d₆) δ 1.15 (br s, 4 H), 1.52 - 1.75 (m, 4 H), 2.42 and 2.43 (2 s, 9 H), 3.04 (m, 8 H), 3.51 (d + m, J = 16.5 Hz, 4 H), 4.01 (d, J = 16.5 Hz, 2 H), 7.35 - 7.53 (m, 8 H), 7.71 (d, J = 8.3 Hz, 4 H), 7.80 (br d, J = 10.5 Hz, 2 H).

G. Synthesis of trans-2,3-Cyclohexano-1,4,7,10,13-pentaazacyclopentadecane

trans-5,6-Cyclohexano-1,10,13-tris(p-toluenesulfonyl)-1,4,7,10,13-pentaazacyclopentadecan-3,8-dione prepared as in Example 2D (1.765 g, 2.32 mmol) was suspended in 1,2-dimethoxyethane (dme, 40 ml) under argon, and the flask was placed in a water bath.

-57-

Lithium aluminum hydride (0.5 M in dme, 55 ml, 27.5 mmol) was added over a 5 min period. Five min later heating with a mantle was started, and reflux began 15 min later. The reaction became almost colorless after a few min of reflux, later turning yellow with white precipitate. Reflux was continued for 43.5 h, and then the reaction mixture was allowed to cool to room temperature. The reaction was quenched by the careful addition of water (0.86 ml) using a water bath for cooling. Five min later, 15% aqueous sodium hydroxide solution (0.86 ml) was added followed by water (2.6 ml). The slight yellowish color largely discharged during this process. One h later, tetrahydrofuran (55 ml) was added and stirring was continued for 2 h. The quenched reaction mixture was filtered. The filtrate was evaporated under reduced pressure and placed on the vacuum line, giving a yellowish-white solid. This solid was dissolved in dichloromethane and filtered, then concentrated to a solid and placed on the vacuum line. It was recrystallized from hot acetonitrile under argon, producing 0.316 g (50% yield) of white needles : mp 112-3 °C (under nitrogen); ¹H NMR (CDCl₃) δ 0.97 (m, 2 H), 1.22 (m, 2H), 1.39 - 1.96 (3 m, 7 H), 2.11 (m, 4 H), 2.49 (m, 2 H), 2.54 - 2.88 (several m, 12 H), 2.94 (m, 2 H); Exact mass (M + H)⁺: calcd, 270.2658; found, 270.2658 (C₁₄H₃₂N₅).

H. Synthesis of [Manganese(II)dichloro(trans-2,3-Cyclohexano-1,4,7,10,13-pentaazacyclopentadecane)]
trans-2,3-Cyclohexano-1,4,7,10,13-

pentaazacyclopentadecane prepared as in Example 2G (301 mg, 1.12 mmol) was added to a hot anhydrous MeOH solution (50 ml) containing anhydrous manganese(II) chloride (140 mg, 1.12 mmol) under a dry nitrogen atmosphere. After refluxing for 2 h, the solution was stirred overnight at room temperature and was then taken to dryness. The white solid was dissolved in warm

-58-

acetone (15 ml) and the solution was filtered. The solution was stripped to dryness and the white solid was washed with ethyl ether. The solid was dried in vacuo to give 0.36 g (82% yield) of product: FAB mass spectrum (NBA) m/z (relative intensity) 394 (M^+ , 1), 359/361 [$(M-Cl)^+$, 100/29]; Anal. calcd. for $C_{14}H_{31}N_5MnCl_2$: C, 42.54; H, 7.91; N, 17.72. Found: C, 42.56; H, 8.17; N, 17.42.

10

Example 3

A. Synthesis of Boc-DAla-Ala-OEt

To a solution of Boc-DAla (25.0 g, 132.1 mmol) in DMF (1450 ml) was added HOBT·H₂O (19.8 g, 129.3 mmol) and EDC·HCl (28.0 g, 146.3 mmol) and the resulting solution was allowed to stir at RT for 30 min. To this solution was added Alanine ethyl ester hydrochloride (20.3 g, 132.1 mmol) and TEA (20.4 ml, 146.3 mmol) and the reaction was allowed to stir for 3 days (for convenience). The DMF was evaporated and the residue was partitioned between water (500 ml) and ethyl acetate (500 ml). The ethyl acetate solution was washed with 1N NaHSO₄ (250 ml), water (250 ml), saturated NaHCO₃ (250 ml), brine (250 ml) and dried over Na₂SO₄. Filtration and concentration afforded 31.7 g (83% yield) of the desired dipeptide as a white foam: ¹H NMR (DMSO-d₆) δ 1.14 (d, J = 7.4 Hz, 3 H), 1.16 (t, J = 7.4 Hz, 3 H), 1.24 (d, J = 7.0 Hz, 3 H), 1.36 (s, 9 H), 3.96 - 4.09 (m, 3 H), 4.17 - 4.22 (apparent quintet, J = 7.4 Hz, 1 H), 6.77 (d, J = 7.7 Hz, 1 H), 8.09 (d, J = 7.0 Hz, 1 H); MS (LRCI, CH₄) m/z (relative intensity) = 317 (5) [$M + C_2H_5$]⁺, 289 (60) [$M + H$]⁺.

B. Synthesis of Boc-Ala-Ala-OH

To a suspension of the dipeptide (15.0 g, 93.6 mmol) in THF (192 ml) was added 0.5 N NaOH solution (192 ml). To

-59-

the resulting solution was added di-t-butylidicarbonate (26.6 g, 121.7 mmol) at once. The pH of the reaction was maintained at -10 for 5 h and the mixture was then allowed to stir overnight. The pH of the reaction was again adjusted to -10 and the solution was extracted with ethyl acetate (2 x 100 ml). The pH of the aqueous layer was adjusted to -3.5 with aqueous potassium bisulfate and this mixture was extracted with ethyl acetate (3 x 100 ml). The combined extracts were dried (MgSO₄), filtered and concentrated to afford 20.7 g (85 % yield) of the desired product as a white powder: ¹H NMR (DMSO-d₆) δ 1.16 (d, J = 6.8 Hz, 3 H), 1.28 (d, J = 7.3 Hz, 3 H), 1.38 (s, 9 H), 3.95 - 4.09 (m, 1 H), 4.20 (quintet, J = 7.3 Hz, 1 H), 6.87 (d, J = 8.0 Hz, 1 H), 8.00 (d, 7.3 Hz, 1 H); MS (HRFAB, NBA - Li) m/z = 267.1557 [M + Li]⁺; 267.1532 calcd for C₁₁H₂₀N₂O₅Li.

C. Synthesis of Dala-Ala-OEt.TFA

The protected dipeptide (31.4 g, 109 mmol) was dissolved in methylene chloride (200 ml) and TFA (66 ml) was added. The resulting solution was allowed to stir for 30 min at RT and concentrated. The residue was coevaporated with methylene chloride (2 x 200 ml), dissolved in ether and oiled out with the addition of excess hexanes. The solvents were decanted and the residue was pumped at high vacuum for 12 h to afford 39.6 g (100 % yield, contains residual TFA) of the desired TFA salt as an orange oil: ¹H NMR (DMSO-d₆) δ 1.16 (t, J = 7.0 Hz, 3 H), 1.28 (d, J = 7.0 Hz, 3 H), 1.34 (d, J = 7.0 Hz, 3 H), 3.86 (bs, 1H), 4.07 (q, J = 7.0 Hz, 2 H), 4.26 (quintet, J = 7.0 Hz, 1 H), 8.21 (bs, 3 H), 8.86 (d, J = 7.4 Hz, 1 H); MS (LRCI, CH₄) m/z (relative intensity) 217 (5) [M + C₂H₅]⁺, 189 (40) [M+H]⁺.

-60-

D. Synthesis of Boc-Ala-Ala-Dala-Ala-OEt

To a solution of Boc-Ala-Ala-OH (20.1 g, 77.2 mmol) in DMF (850 ml) was added HOBT·H₂O (13.1 g, 85.4 mmol) and EDC·HCl (16.4 g, 85.4 mmol). To this solution was added
5 Dala-Ala-OEt·TFA (23.3 g, 77.2 mmol) followed by TEA (11.9 ml, 85.4 mmol) and the resulting mixture was stirred for 12 h thereafter. The DMF was evaporated and the residue was dissolved in ethyl acetate (300 ml) and washed with 1 N potassium bisulfate (150 ml), water (150
10 ml), saturated sodium bicarbonate (150 ml) and brine (150 ml). The ethyl acetate layer was dried (MgSO₄), filtered and concentrated to half volume and crystallization was allowed to proceed. Isolation by filtration afforded 20.5 g (62 % yield) of the desired
15 tetrapeptide as a white solid: ¹H NMR (DMSO-d₆) δ 1.13 (d, J = 7.0 Hz, 3 H), 1.17 (two coincidental d, J = 7.0 Hz, 6 H), 1.25 (d, J = 7.4 Hz, 3 H), 3.91 - 4.30 (m, 6 H), 6.87 (d, 7.0 Hz, 1 H), 7.92 (d, J = 6.3 Hz, 1 H), 8.07 (d, J = 7.3 Hz, 1 H), 8.09 (d, J = 6.6 Hz, 1 H);
20 MS (HRFAB, NBA - Li) m/z = 437.2600 [M + Li]⁺; 437.2588 calcd for C₁₉H₃₄N₄O₇Li.

E. Synthesis of Boc-Ala-Ala-Dala-Ala-OH

A solution of Boc-Ala-Ala-Dala-Ala-OEt (10.9 g, 25.3
25 mmol) in methanol (100 ml) was treated with 2.5 M sodium hydroxide (20.0 ml, 50.0 mmol) and the resulting solution was allowed to stir for 2 h at RT. At this time the pH of the solution was lowered to ~3 with the addition of aqueous potassium bisulfate and the
30 resulting mixture was extracted with ethyl acetate (3 x 100 ml). The combined extracts were dried (MgSO₄), filtered and concentrated to afford 6.8 g (67 % yield) of the desired acid as a white solid: ¹H NMR (DMSO-d₆) δ 1.17 (d, J = 7.2 Hz, 3 H), 1.20 (two coincidental d, J =
35 7.1 Hz, 6 H), 1.28 (d, J = 1.3 Hz, 3 H), 1.38 (s, 9 H), 3.90 - 4.00 (m, 1 H), 4.17 - 4.30 (m, 3 H), 6.93 (d, J =

-61-

6.7 Hz, 1 H), 7.96 (d, J = 6.7 Hz, 1 H), 8.04 (d, J = 7.4 Hz, 1 H), 8.07 (d, J = 7.8 Hz, 1 H); MS (HRFAB, NBA - Li) m/z = 409.2331 [M + Li]⁺; 409.2353 calcd for C₁₇H₃₀N₄O₇Li.

5

F. Synthesis of Boc-Ala-Ala-DAla-Ala-DAla-OBzl

To a solution of Boc-Ala-Ala-DAla-Ala-OH (6.5 g, 16.3 mmol) in DMF (180 ml) was added HOBT·H₂O (2.86 g, 18.7 mmol) and EDC·HCl (3.58 g, 18.7 mmol). The resulting solution was allowed to stir for 15 min at RT and treated with DAla-OBzl p-toluenesulfonate salt (6.57 g, 18.7 mmol) and TEA (2.6 ml, 18.7 mmol). This mixture was allowed to stir for 12 h thereafter. The DMF was evaporated and the residue was partitioned between ethyl acetate (300 ml) and water (300 ml). The ethyl acetate layer was washed with 1 N potassium bisulfate (150 ml), water (150 ml), saturated sodium bicarbonate (150 ml) and brine (150 ml). The ethyl acetate layer was then dried (MgSO₄), filtered and concentrated to afford 9.0 g (100 % yield) of the desired compound as a white powder: ¹H NMR (DMSO-d₆) δ 1.17 (d, J = 7.3 Hz, 3 H), 1.21 (two coincidental d, J = 7.0 Hz, 6 H), 1.22 (d, J = 7.0 Hz, 3 H), 1.32 (d, J = 7.3 Hz, 3 H), 1.37 (s, 9 H), 3.90 - 4.09 (m, 1 H), 4.18 - 4.34 (m, 4 H), 5.13 (ABq, J = 12.7, Δν = 10.5 Hz, 2 H), 6.94 (d, J = 7.3 Hz, 1 H), 7.30 - 7.41 (m, 5 H), 7.97 (d, J = 7.0 Hz, 1 H), 8.10 - 8.18 (m, 2 H), 8.25 (d, J = 6.9 Hz, 1 H); MS (HRFAB, NBA - Li) m/z = 570.3140 [M + Li]⁺; 570.3115 calcd for C₂₇H₄₁N₅O₈Li.

30

G. Synthesis of Ala-Ala-DAla-Ala-DAla·HCl

Boc-Ala-Ala-DAla-Ala-DAla-OEt (10.4 g, 18.7 mmol) was dissolved in acetic acid (225 ml) and treated with concentrated hydrochloric acid (75 ml). The resulting solution was allowed to stir at RT for 14 h thereafter. At this time the reaction was concentrated, coevaporated

35

-62-

with water (50 ml) and azeotropically dried by toluene coevaporation (2 x 100 ml) to afford 7.8 g (96 % yield) of the deprotected pentapeptide hydrochloride as a white powder: ^1H NMR (D_2O) δ 1.29 - 1.39 (m, 12H), 1.47 (d, J = 7.0 Hz, 3 H), 4.06 (q, J = 7.0 Hz, 1 H), 4.18 - 1.38 (m, 4 H); MS (LRFAB, NBA - HCl) 374 $[\text{M} + \text{H}]^+$.

H. Synthesis of Cyclo-(Ala-Ala-DAla-Ala-DAla-)

To a solution of Ala-Ala-DAla-Ala-DAla·HCl (7.8 g, 19.0 mmol) in DMF (2400 ml) at -40°C was added DPPA (6.29 g, 22.8 mmol) and enough TEA to adjust the "pH" to -8 (measured by spotting the reaction mixture on moistened hydrion paper). This solution was allowed to stand at -23°C for 48 hours and at 8°C for 48 hours. During this time the "pH" was again maintained at -8 with the periodic addition of TEA. At the end of this period the reaction mixture was poured into water (2400 ml) and stirred with mixed-bed ion exchange resin (1200 g) for 6 h. The resin was removed by filtration and the filtrate was concentrated to a volume of ~ 100 ml. Ether (500 ml) was added and the precipitated white solid was isolated by filtration and washed with more ether (250 ml). The solid was then triturated by stirring with THF (100 ml) for 12 h (to remove traces of DMF), filtered and thoroughly dried to afford 3.15 g (47 % yield) of the desired cyclic peptide as a fine white powder: ^1H NMR ($\text{DMSO}-d_6$) δ 1.08 - 1.25 (m, 12 H), 1.24 (d, J = 7.3 Hz, 3 H), 4.00 - 4.10 (m, 1 H), 4.26 - 4.30 (m, 2 H), 4.34 (q, J = 7.2 Hz, 1 H), 4.41 (q, J = 7.6 Hz, 1 H), 7.58 (d, J = 7.0 Hz, 1 H), 7.83 (d, J = 8.4 Hz, 1 H), 8.22 (d, J = 6.2 Hz, 1 H), 8.33 (d, J = 7.81, 1 H), 8.49 (d, J = 6.8 Hz, 1 H); MS (HRFAB, NBA - HCl) m/z 356.1989 $(\text{M} + \text{H})^+$; 356.1934 calcd for $\text{C}_{15}\text{H}_{25}\text{N}_5\text{O}_5$ $(\text{M} + \text{H})^+$.

-63-

I. Synthesis of (2S, 5R, 8S, 11R, 14S)-Pentamethyl-1,4,7,10,13-pentaazacyclopentadecane

- To a stirred suspension of cyclo-(Ala-Ala-Dala-Ala-Dala-) (3.10 g, 8.70 mmol) in THF (70 ml) at RT was added
- 5 lithium aluminum hydride (108 ml of a 1.0 M solution in THF, 108 mmol). The resulting mixture was stirred at RT for 2 h and heated to reflux for 16 h thereafter. The mixture was then cooled to -20 °C and quenched with the dropwise addition of saturated sodium sulfate (~30 ml).
- 10 The resulting mixture was concentrated to a dry white powder and this powder was triturated with ether (2 x 150 ml). The combined triturates were concentrated and recrystallized from acetonitrile to afford 1.10 g (44 % yield) of the desired ligand as a white solid: ¹H NMR
- 15 (CDCl₃) δ 0.96 (d, J = 5.2 Hz, 3 H), 1.00 (two coincidental d, J = 5.0 Hz, 6 H), 1.02 (two coincidental d, J = 5.0 Hz, 6 H), 1.30 - 1.55 (bm, 2 H), 1.85 - 2.15 (bs, 3 H), 2.05 - 2.19 (m, 5 H), 2.42 - 3.00 (complex m, 12 H); MS (HRFAB, NBA - HCl) m/z = 286.3013 (M +
- 20 H)⁺; 286.2971 calcd for C₁₅H₃₆N₅.

J. Synthesis of [Manganese(II)dichloro-(2S, 5R, 8S, 11R, 14S)-Pentamethyl-1,4,7,10,13-pentaazacyclopentadecane

- 25 To a stirred solution of anhydrous MnCl₂ (79.0 mg, 0.62 mmol) in hot ethanol (5 ml) was added (2S, 5R, 8S, 11R, 14S)-pentaazacyclopentadecane (177 mg, 0.62 mmol). The solution was refluxed for 1 h and stirred at RT for an additional 16 h. The solution was filtered through
- 30 celite, concentrated to half volume and treated with ether (30 ml). The white crystals were isolated by filtration and dried in vacuo to afford 187 mg (73 % yield) of the complex as a white solid: MS (LRFAB, NBA) m/z (relative intensity) = 410 (5) [M]⁺, 375/377
- 35 (100/30) [M - Cl]⁺; Anal. calcd. for C₁₅H₃₅N₅MnCl₂: C,

-64-

43.75; H, 8.57; N, 17.01. Found: C, 43.74; H, 8.60; N, 16.97.

Example 4

5 A. Synthesis of N-(p-toluenesulfonyl)-(R,R)-1,2-diaminocyclohexane

To a stirred solution of (R,R)-1,2-diaminocyclohexane (300 g, 2.63 mole) in CH_2Cl_2 (5.00 l) at -10°C was added a solution of p-
10 toluenesulfonylchloride (209 g, 1.10 mole) in CH_2Cl_2 (5.00 l) dropwise over a 7 h period, maintaining the temp at -5 to -10°C . The mixture was allowed to warm to room temp while stirring overnight. The mixture was concentrated in vacuo to a volume of 3 l and the white
15 solid was removed by filtration. The solution was then washed with H_2O (10 x 1 l) and was dried over MgSO_4 . Removal of the solvent in vacuo gave 286 g (97.5 % yield) of the product as a yellow crystalline solid: ^1H NMR (CDCl_3) δ 0.98 - 1.27 (m, 4 H), 1.54 - 1.66 (m, 2 H), 1.81 - 1.93 (m, 2 H), 2.34 (dt, $J = 4.0$, 10.7 Hz, 1 H), 2.42 (s, 3 H), 2.62 (dt, $J = 4.2$, 9.9 Hz, 1 H), 7.29 (d, $J = 8.1$ Hz, 2 H), 7.77 (d, $J = 8.3$ Hz, 2 H); MS (LRFAB - DTT - DTE) m/z 269 $[\text{M} + \text{H}]^+$.

25 B. Synthesis of N-(p-toluenesulfonyl)-N'-(Boc)-(R,R)-1,2-diaminocyclohexane

To a stirred solution of N-(p-toluenesulfonyl)-(R,R)-1,2-diaminocyclohexane prepared as in Example 1A (256 g, 0.955 mole) in THF (1.15 l) was added a 1 N
30 solution of aqueous NaOH (1.15 l, 1.15 mole). Di-*t*-butyldicarbonate (229 g, 1.05 mole) was then added and the resulting mixture was stirred overnight. The layers were separated and the aqueous layer was adjusted to pH 2 with 1 N HCl and saturated with NaCl. The aqueous
35 solution was then extracted with CH_2Cl_2 (2 x 500 ml) and the extracts and THF layer were combined and dried over

-65-

MgSO₄. The solvent was removed in vacuo to give a yellow solid. The crude product was purified by crystallization from a THF-ether-hexanes mixture to give 310 g (88.1% yield) of the product as a white

- 5 crystalline solid: mp: 137 - 139 °C; ¹H NMR (CDCl₃) δ 1.04 - 1.28 (m, 4 H), 1.44 (s, 9 H), 1.61 - 1.69 (m, 2 H), 1.94 - 2.01 (m, 2 H), 2.43 (s, 3 H), 2.86 (brs, 1 H), 3.30 (br d, J = 9.6 Hz, 1 H), 4.37 (br d, J = 6.7 Hz, 1 H), 5.48 (br d, J = 4.6 Hz, 1 H), 7.27 (d, J = 9.7
10 Hz, 2 H), 7.73 (d, J = 8.1 Hz, 2 H); MS (LRFAB, NBA - Li) m/z 375 [M + Li]⁺.

C. Synthesis of Boc-(R,R)-Cyc(Ts)-gly-OMe

- To a stirred solution of N-(p-toluenesulfonyl)-N' -
15 (Boc)-(R,R)-1,2-diaminocyclohexane prepared as in Example 1B (310 g, 0.841 mole) in anhydrous DMF (3.11 l) at 0 °C was added NaH (37.4 g - 60 % in oil, 0.934 mole) in portions and the resulting mixture was stirred for 30 min. Methyl bromoacetate (142 g, 0.925 mole) was then
20 added dropwise over 45 min and the mixture was allowed to warm to room temp while stirring overnight. After stirring for 17 h, the solvent was removed in vacuo and the residue was dissolved in ethyl acetate (3 l) and H₂O (1 l). The ethyl acetate solution was washed with
25 saturated NaHCO₃ (1 l), saturated NaCl (500 ml) and was dried over MgSO₄. The solvent was removed in vacuo and the resulting oil was dissolved in ether. Crystallization by the addition of hexanes gave 364 g (98 % yield) of the product (TLC (98:2 CHCl₃-
30 MeOH/silica gel/UV detn) showed that the product contained about 5% starting material) as colorless needles: mp of pure sample 151 - 2 °C; ¹H NMR (CDCl₃) δ 1.11 - 1.22 (m, 4 H), 1.45 (s, 9 H), 1.64 - 1.70 (m, 3 H), 2.16 - 2.19 (m, 1 H), 2.43 (s, 3 H), 3.34 - 3.40 (m, 2 H), 3.68 (s, 3 H), 4.06 (ABq, J = 18.5 Hz, Δν = 155
35 Hz, 2H), 4.77 (br s 1 H), 7.30 (d, J = 8.3 Hz, 2 H),

7.82 (d, $J = 8.3$ Hz, 2 H); MS (LRFAB, DTT - DTE) m/z 441 $[M + H]^+$.

D. Synthesis of Boc-(R,R)-Cyc(Ts)-Gly-OH

5 To a stirred solution of impure Boc-(R,R)-Cyc(Ts)-Gly-OMe prepared as in Example 1C (217 g, 0.492 mole) in MeOH (1.05 l) was slowly added a 2.5N solution of aqueous NaOH (295 ml, 0.737 mole) and the resulting solution was stirred for 2 h. The solvent was removed
10 in vacuo and the residue was dissolved in H_2O (1.5 l). The solution was filtered to remove a small amount of solid and was washed with ether (7 x 1 l) to remove the impurity (compound 1B) which upon drying of the combined washes over $MgSO_4$ and removal of the solvent in vacuo
15 resulted in recovery of 8.37 g. The pH of the aqueous solution was then adjusted to 2 with 1 N HCl and the product was extracted with ethyl acetate (3 x 1 l). The extracts were combined, washed with saturated NaCl (500 ml) and dried over $MgSO_4$. The solvent was removed
20 in vacuo and the residual ethyl acetate removed by coevaporation with ether (500 ml) and then CH_2Cl_2 (500 ml) to give 205 g (97.6 % yield) of the product as a white foam: 1H NMR ($CDCl_3$) δ 1.15 - 1.22 (m, 4 H), 1.48 (s, 9 H), 1.55 - 1.68 (m, 3 H), 2.12 - 2.15 (m, 1 H),
25 2.43 (s, 3 H), 3.41 - 3.49 (m, 2 H), 3.97 (ABq, $J = 17.9$ Hz, $\Delta \nu = 69.6$ Hz, 2 H), 4.79 (br s, 1 H), 7.31 (d, $J = 8.1$ Hz, 2 H), 7.77 (d, $J = 8.3$ Hz, 2 H), 8.81 (br s, 1 H); MS (LRFAB, NBA - Li) m/z 433 $[M + Li]^+$.

30 E. Synthesis of N-(p-toluenesulfonyl)-(S,S)-1,2-diaminocyclohexane

To a stirred solution of (S,S)-1,2-diaminocyclohexane (300 g, 2.63 mole) in CH_2Cl_2 (5.00 l) at $-10^\circ C$ was added a solution of p-
35 toluenesulfonylchloride (209 g, 1.10 mole) in CH_2Cl_2 (5.00 l) dropwise over a 8 h period, maintaining the

-67-

temp at -5 to -10 °C. The mixture was allowed to warm to RT while stirring overnight. The mixture was concentrated in vacuo to a volume of 3 l and the white solid was removed by filtration. The solution was then washed with H₂O (10 x 1 l) and was dried over MgSO₄. Removal of the solvent in vacuo gave 289 g (98.3 % yield) of the product as a yellow crystalline solid: ¹H NMR (CDCl₃) δ 0.98 - 1.27 (m, 4 H), 1.55 - 1.66 (m, 2 H), 1.81 - 1.94 (m, 2 H), 2.32 (dt, J = 4.0, 10.9 Hz, 1 H), 2.42 (s, 3 H), 2.61 (dt, J = 4.0, 9.9 Hz, 1 H), 7.30 (d, J = 7.9 Hz, 2 H), 7.77 (d, J = 8.3 Hz, 2 H); MS (LRFAB,GT - HCl) m/z 269 [M + H]⁺.

15 F. Synthesis of N-(p-toluenesulfonyl)-N'-(Boc)-(S,S)-1,2-diaminocyclohexane

To a stirred solution of N-(p-toluenesulfonyl)-(S,S)-1,2-diaminocyclohexane prepared as in Example 1E (289 g, 1.08 mole) in THF (1.29 l) was added a 1 N solution of aqueous NaOH (1.29 l, 1.29 mole). Di-t-butylidicarbonate (258 g, 1.18 mole) was then added and the resulting mixture was stirred overnight. The solid was removed by filtration and washed with THF. The THF layer was separated and the aqueous layer was adjusted to pH 2 with 1 N HCl and saturated with NaCl. The aqueous solution was then extracted with CH₂Cl₂ (2 x 500 ml) and the extracts and THF layer were combined, washed with saturated NaCl (500 ml) and dried over MgSO₄. The solvent was removed in vacuo to give a yellow slurry. Crystallization with the addition of ether gave 364 g (91.9 % yield) of the product as colorless needles: mp 137 - 139 °C; ¹H NMR (CDCl₃) δ 1.06 - 1.31 (m, 4 H), 1.44 (s, 9 H), 1.60 - 1.69 (m, 2 H), 1.95 - 1.99 (m, 2 H), 2.42 (s, 3 H), 2.86 (br s, 1 H), 3.30 (br d, J = 2.6 Hz, 1 H), 4.41 (br d, J = 7.3 Hz, 1 H), 5.54 (br d, J = 5.4

-68-

Hz, 1 H), 7.28 (d, J = 8.1 Hz, 2 H), 7.73 (d, J = 8.3 Hz, 2 H); MS (LRFAB, NBA - HCl) m/z 369 [M + H]⁺.

G. Synthesis of Boc-(S,S)-Cyc(Ts)-gly-OMe

- 5 To a stirred solution of N-(p-toluenesulfonyl)-N'-(Boc)-(S,S)-1,2-diaminocyclohexane prepared as in Example 1F (364 g, 0.989 mole) in anhydrous DMF (3.66 l) at 0 °C was added NaH (47.4 g - 60 % in oil, 1.19 mole) in portions and the resulting mixture was stirred for
- 10 1.5 h. The mixture was warmed to room temp and stirred an additional 30 min and then cooled back to 0 °C. Methyl bromoacetate (189 g, 1.24 mole) was added dropwise over 30 min and the mixture was allowed to warm to RT while stirring overnight. After stirring for 17
- 15 h, the solvent was removed in vacuo and the residue was dissolved in a mixture of ethyl acetate (3 l) and H₂O (1 l). The layers were separated and the ethyl acetate solution was washed with saturated NaHCO₃ (1 l), H₂O (1 l), saturated NaCl (2 x 500 ml) and was dried over
- 20 MgSO₄. The solvent was removed in vacuo and the resulting oil was dissolved in ether. Crystallization by the addition of hexanes gave 290 g of the crude product as yellow needles. Another 180 g was recovered from the filtrate as an oil. TLC (98:2 CHCl₃-
- 25 MeOH/silica gel/UV detn) showed that both the solid and the oil contained starting material. ¹H NMR (CDCl₃) δ 1.06 - 1.29 (m, 4 H), 1.44 (s, 9 H), 1.58 - 1.66 (m, 3 H), 2.17 - 2.19 (m, 1 H), 2.43 (s, 3 H), 3.28 - 3.43 (m, 2 H), 3.68 (s, 3 H), 4.25 (ABq, J = 18.5 Hz, Δν = 115
- 30 Hz, 2H), 4.76 (br s 1 H), 7.31 (d, J = 8.3 Hz, 2 H), 7.83 (d, J = 8.3 Hz, 2 H); MS (LRFAB, NBA - Li) m/z 447 [M + H]⁺.

H. Synthesis of Boc-(S,S)-Cyc(Ts)-Gly-OH

- 35 To a stirred solution of impure Boc-(S,S)-Cyc(Ts)-Gly-OMe prepared as in Example 1G (197 g, 0.447 mole) in

-69-

- MeOH (925 ml) was slowly added a 2.5N solution of aqueous NaOH (268 ml, 0.670 mole) and the resulting solution was stirred for 2 h. The solvent was removed in vacuo and the residue was dissolved in H₂O (1 l).
- 5 The solution was washed with ether (4 x 1 l) to remove the impurity (compound 1F) which upon drying of the combined washes over MgSO₄ and removal of the solvent in vacuo resulted in recovery of 14.8 g. The pH of the aqueous solution was then adjusted to 2 with 1 N HCl and
- 10 the product was extracted with ethyl acetate (3 x 1 l). The extracts were combined, washed with saturated NaCl and dried over MgSO₄. The solvent was removed in vacuo to give 171 g (89.7 % yield) of the product as an oil which crystallized on standing: ¹H NMR (CDCl₃) δ 1.10 -
- 15 1.22 (m, 4 H), 1.45 (s, 9 H), 1.55 - 1.68 (m, 3 H), 2.13 - 2.16 (m, 1 H), 2.43 (s, 3 H), 3.39 - 3.41 (m, 2 H), 4.00 (ABq, J = 18.1 Hz, Δ^u = 80.4 Hz, 2 H), 4.82 (br s, 1 H), 7.31 (d, J = 8.3 Hz, 2 H), 7.75 (d, J = 8.3 Hz, 2 H), 9.28 (br s, 1 H); MS (LRFAB, NBA - Li) m/z 433 [M +
- 20 Li]⁺.

I. Synthesis of Boc-(S,S)-Cyc(Ts)-Gly-Gly-OEt

- To a stirred solution of Boc-(S,S)-Cyc(Ts)-Gly-OH prepared as in Example 1H (26.7 g, 62.5 mmole) in
- 25 degassed anhydrous DMF (690 ml) was added HOBT (10.1 g, 75.0 mmole) and EDC-HCl (14.4 g, 75.0 mmole). After the resulting solution was stirred for 30 min, glycine ethyl ester hydrochloride (9.60 g, 68.8 mmole) was added and the pH adjusted to 8 with TEA. After stirring for 2.75
- 30 days the solvent was removed in vacuo. The residue was dissolved in a mixture of ethyl acetate (1 l) and H₂O (1 l) and the layers were separated. The aqueous layer was extracted with ethyl acetate (1 l) and the extracts were combine. The ethyl acetate solution was washed with 0.1
- 35 N HCl (1 l), saturated NaHCO₃ (1 l), saturated NaCl (500 ml) and was dried over MgSO₄. The solvent was removed

-70-

in vacuo to give 30.2 g (94.4 % yield) of the product as a white foam: ^1H NMR (CDCl_3) δ 1.19 - 1.23 (m, 3 H), 1.28 (t, $J = 7.05$ Hz, 3 H), 1.42 (s, 11 H), 1.63 - 1.71 (m, 2 H), 2.16 - 2.18 (m, 1 H), 2.43 (s, 3 H), 3.50 - 3.57 (m, 2 H), 3.83 (ABq, $J = 17.7$ Hz, $\Delta \nu = 35.7$ Hz, 2 H), 4.01 (dABq, $J = 6.05$, 17.92 Hz, $\Delta \nu = 28.9$ Hz, 2 H), 4.20 (q, $J = 7.3$ Hz, 2 H), 4.88 (br s, 1 H), 7.31 (d, $J = 8.3$ Hz, 2 H), 7.36 (br s, 1 H), 7.73 (d, $J = 8.3$ Hz, 2 H); MS (LRFAB, NBA - HCl) m/z 512 $[\text{M} + \text{H}]^+$.

10

J. Synthesis of (S,S)-Cyc(Ts)-Gly-Gly-OEt TFA salt

To a stirred solution of Boc-(S,S)-Cyc(Ts)-Gly-Gly-OEt prepared as in Example 1I (30.1 g, 58.8 mmole) in CH_2Cl_2 (265 ml) was added TFA (63 ml) and the resulting solution was stirred for 30 minutes. The solvent was removed in vacuo and residual TFA was coevaporated with CH_2Cl_2 (2 x 1 l) and ether (1 l). The oil was then triturated with ether (2 x 1 l) and the ether decanted. The resulting foam was dried in vacuo to give 33.7 g (assumed quantitative yield) of the product as a tan powder: ^1H NMR (CDCl_3) δ 0.96 - 1.23 (m, 4 H), 1.25 (t, $J = 7.3$ Hz, 3 H), 1.51 - 1.66 (m, 3 H), 2.12 - 2.26 (m, 1 H), 2.41 (s, 3 H), 2.98 - 3.10 (brs, 1 H), 3.67 - 3.71 (m, 1 H), 4.04 (ABq, $J = 17.7$ Hz, $\Delta \nu = 154$ Hz, 2 H), 4.04 (d, $J = 4.4$ Hz, 2 H), 4.17 (q, $J = 7.3$ Hz, 2 H), 7.29 (d, $J = 8.3$ Hz, 2 H), 7.70 (d, $J = 8.3$ Hz, 2 H), 8.04 (br s, 1 H), 8.14 (br s, 3 H) MS (LRFAB, NBA - HCl) m/z 412 $[\text{M} + \text{H}]^+$.

K. Synthesis of Boc-(R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly-OEt

To a stirred solution of Boc-(R,R)-Cyc(Ts)-Gly-OH prepared as in Example 1D (25.1 g, 58.8 mmole) in degassed anhydrous DMF (650 ml) was added HOBT (9.54 g, 70.6 mmole) and EDC-HCl (13.5 g, 70.6 mmole). After the resulting solution was stirred for 30 min (S,S)-Cyc(Ts)-

-71-

Gly-Gly-OEt TFA salt prepared as in Example 1J (33.6 g, 58.8 mmole) was added and the pH was adjusted to 8 with TEA. After stirring for 2.75 days, the solvent was removed in vacuo. The residue was dissolved in a mixture of ethyl acetate (1 l) and H₂O (1 l) and the layers were separated. The ethyl acetate solution was washed with 0.1 N HCl (2 x 1 l), saturated NaHCO₃ (2 x 1 l), saturated NaCl (500 ml) and was dried over MgSO₄. The solvent was removed in vacuo to give 47.5 g (98.4 % yield) of the product as a tan foam: ¹H NMR (CDCl₃) δ 1.12 - 1.83 (m, 26 H), 2.21 - 2.24 (m, 2 H), 2.42 (s, 3 H), 2.43 (s, 3 H), 3.36 - 3.51 (br s, 2 H), 3.68 - 3.96 (m, 6 H), 4.00 (d, J = 5.4 Hz, 2 H), 4.19 (q, J = 7.1 Hz, 2 H), 4.72 (br s, 1 H), 6.78 (br s, 1 H), 7.31 (d, J = 8.1 Hz, 4 H), 7.46 (br s, 1 H), 7.79 (m, 4 H); MS (LRFAB, NBA - HCl) m/z 820 [M + H]⁺.

L. Synthesis of Boc-(R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly-OH

To a stirred solution of Boc-(R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly-OEt prepared as in Example 1K (47.4 g, 57.8 mmole) in MeOH (240 ml) was added a 2.5 N solution of aqueous NaOH (34.7 ml, 86.7 mmole) and the resulting solution was stirred for 2 h. The solvent was removed in vacuo and the residue was dissolved in H₂O (1 l). The aqueous solution was washed with ether (2 x 1 l) and the pH was adjusted to 2 with 1 N HCl. The solution was then saturated with NaCl and extracted with ethyl acetate (3 x 1 l). The combined extracts were dried over MgSO₄ and the solvent was removed in vacuo. The residual ethyl acetate was removed by coevaporation with CH₂Cl₂ and the resulting foam was dried in vacuo to give 45.7 g (99.7 % yield) of the product as a tan powder: ¹H NMR (CDCl₃) δ 1.16 - 1.75 (m, 23 H), 2.13 - 2.17 (m, 2 H), 2.41 (s, 3 H), 2.42 (s, 3 H), 3.49 - 4.16 (m, 10 H), 4.53 (br s, 1 H), 7.01 (br s, 1 H), 7.30 (d,

-72-

J = 8.1 Hz, 4 H), 7.40 (br s, 1 H), 7.79 (d, J = 8.1 Hz, 2 H), 7.86 (d, J = 7.7 Hz, 2 H), 10.40 (br s, 1 H); MS (LRFAB, NBA - HCl) m/z 792 [M + H]⁺.

5 M. Synthesis of (R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly-OH TFA salt

To a stirred solution of Boc-(R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly-OH prepared as in Example 1L (45.5 g, 57.5 mmole) in CH₂Cl₂ (260 ml) was added TFA (60 ml).
10 The resulting solution was stirred for 30 min and the solvent was removed in vacuo. Residual TFA was removed by coevaporation with CH₂Cl₂ (3 x 1 l) and trituration of the resulting foam with ether (1 l, 2 x 750 ml), decanting the ether each time. After dessication in
15 vacuo, 47.4 g (100 % yield) of the product was obtained as an off white powder: ¹H NMR (CDCl₃) δ 1.05 - 1.31 (m, 9 H), 1.48 - 1.63 (m, 5H), 2.11 - 2.21 (m, 2 H), 2.40 (s, 3 H), 2.42 (s, 3 H), 3.25 (br s, 1 H), 3.60 - 3.80 (m, 3 H), 3.83 - 4.19 (m, 6 H), 6.94 (br s, 1 H),
20 7.31 (m, 4 H), 7.69 (m, 4 H), 7.83 (br s, 3 H), 13.17 (br s, 2 H); MS (LRFAB, DTT - DTE) m/z 692 [M + H]⁺.

N. Synthesis of Cyclo-[(R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly-]

25 To a stirred solution of (R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly-OH TFA salt prepared as in Example 1M (32.2 g, 40.0 mmole) in degassed anhydrous DMF (10.0 l) at -78° C was added DPPA (13.4 g, 48.8 mmole). The pH of the solution was then adjusted to 8 with TEA and the
30 solution was allowed to stand for 6 h at -78° C. The pH was readjusted to 8 with TEA and the solution was warmed to -45° C for 24 h. After readjusting the pH as before, the solution was allowed to warm to -40° C for 24 h. The pH was adjusted as before and the solution was
35 allowed to stand at -20° C for 24 h. The pH was readjusted as before and the solution was allowed to

-73-

warm to 2° C over 24 h. The pH had dropped only slightly. The pH was readjusted as before and the solution was allowed to stand at 2° C for another 24 h after which time the pH had not changed. The solution
5 was divided equally among 6 - 4 l beakers and H₂O (1.1 l) was added to each. Then added a total of 5.00 kg mixed-bed ion exchange resin to the solution (divided equally among the 6 beakers) and stirred the mixtures for 6 h. The resin was then filtered and washed with DMF. The
10 solvent was then removed in vacuo and the solid residue was dissolved in MeOH (100 ml) and filtered to remove finely divided solids. The solution was then concentrated in vacuo to a volume of 25 ml and ether was added periodically as the crystallization proceeded to
15 give 22.2 g (82.5 % yield) of the product as colorless needles; mp 190 - 200° C; ¹H NMR (CDCl₃) δ 0.87 - 2.13 (m, 16 H), 2.41 (s, 3 H), 2.45 (s, 3 H), 3.56 - 3.97 (m, 10 H), 6.66 (br s, 1 H), 7.18 (br s, 1 H), 7.34 (d, J = 8.1 Hz, 4 H), 7.65 (br s, 1 H), 7.71 (d, J = 7.3 Hz, 2
20 H), 7.39 (d, J = 7.3 Hz, 2 H); MS (LRFAB, NBA - Li) m/z 680 [M + Li]⁺.

O. Synthesis of 2,3-(R,R)-8,9-(S,S)-Bis-cyclohexano-1,4,7,10,13-pentaazacyclopentadecane

25 To a stirred solution of Cyclo-[(R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly] prepared as in Example 1N (19.4 g, 28.8 mmole) in anhydrous THF (475 ml) was added a solution of 1.0 M LiAlH₄ in THF (345 ml, 345 mmole) dropwise over 30 min. The yellow homogeneous solution
30 was refluxed for 20 h (by which time it had become heterogeneous) and was then cooled to 0° C. The mixture was then quenched by the dropwise addition of a 10 % NaSO₄ solution (50 ml) while cooling in an ice bath. The solids were removed by filtration under an Ar blanket
35 and the THF was removed in vacuo to give an oil which rapidly crystallized. The solids were then refluxed

-74-

- with anhydrous THF (1 l) for 1 h and the mixture was filtered and the solvent removed in vacuo as before. The solids were then refluxed with a mixture of THF (1 l) and MeOH (500 ml) for 1 h and worked up as before.
- 5 The residues from the extractions were then dissolved in anhydrous THF, combined and solids were removed by filtration. The solvent was removed in vacuo and the yellow foam dried by azeotroping H₂O with toluene (1.75 l) in vacuo at 90° C. Then refluxed the solids with
- 10 hexanes (1 l) for 30 min and transferred the hot solution to a tared flask and removed the solvent in vacuo to give 6.1 g of an oil which crystallized on standing. The remaining solids were refluxed with hexanes as before and obtained 1.4 g of an oil which
- 15 crystallized on standing. The solids were then dissolved in MeOH and toluene (1 l) was added. The solvent was removed in vacuo and any remaining H₂O was removed by azeotroping with toluene (1 l) and then hexanes (3 x 1 l). The resulting fine powder was
- 20 refluxed with hexanes (1 l) for 2 h under argon and filtered into a tared flask. The solvent was removed in vacuo to give 1.7 g oil which crystallized on standing. The crystalline residues from the 3 extracts were dissolved in hexanes and combined. A small amount of
- 25 haziness was removed by filtration and the solution was concentrated to give 5.3 g (57 % yield) of product as a pale yellow crystalline solid. Recrystallization from acetonitrile gave 4.47 g (48.0 % yield) of a colorless crystalline solid: mp 107 - 8° C; ¹H NMR (CDCl₃) δ 0.95 -
- 30 1.01 (m, 4 H), 1.19 - 1.24 (m, 4 H), 1.70 - 1.73 (m, 4 H), 1.97 (br s, 5 H), 2.08 - 2.14 (m, 8 H), 2.49 - 2.68 (m, 6 H), 2.74 - 2.80 (m, 2 H), 2.85 - 2.90 (m, 2 H), 2.94 - 2.99 (m, 2 H); MS (LRFAB, NBA) m/z 324 [M + H]⁺; Anal. calcd. for C₁₈H₂₇N₃: C, 66.83; H, 11.53; N, 21.65.
- 35 Found: C, 66.80; H, 11.44; N, 21.71.

-75-

P. Synthesis of [Manganese(II) dichloro (2,3-(R,R)-8,9-(S,S)-Bis-cyclohexano-1,4,7,10,13-pentaazacyclopentadecane] chloride

To a stirred solution of anhydrous MnCl_2 (1.67 g, 5 13.3 mmole) in hot methanol (120 ml) was added 2,3-(R,R)-8,9-(S,S)-bis-cyclohexano-1,4,7,10,13-pentaazacyclopentadecane prepared as in Example 10 (4.30 g, 13.3 mmole) and the solution was refluxed and then stirred at room temp overnight. Crystallization from 10 ether gave 5.11 g (85.6 % yield) of the product as an off-white crystalline solid: MS (LRFAB, NBA) m/z (relative intensity) 448 (2) $[\text{M}]^+$, 413/415 (100,33) $[\text{M} - \text{Cl}]^+$; Anal. calcd. for $\text{C}_{18}\text{H}_{27}\text{N}_5\text{MnCl}_2$: C, 48.11; H, 8.30; N, 15.59; Cl, 15.78. Found: C, 48.18; H, 8.32; N, 15.56; 15 Cl, 15.75.

Example 5

A. Synthesis of Boc-DSer(OBzl)-OMe

To Boc-DSer(OBzl)-OH (15.0 g, 50.8 mmol) in ACN (250 20 ml) was added Cs_2CO_3 (33.0 g, 102 mmol) and methyl iodide (6.32 ml, 102 mmol) and the resulting mixture was stirred at RT for 3 h thereafter. At this time the reaction was filtered and concentrated. The residue was partitioned between water (250 ml) and ethyl acetate 25 (250 ml). The ethyl acetate layer was dried (MgSO_4), filtered and concentrated to afford 15.0 g (95 % yield) of the desired methyl ester as a white solid: ^1H NMR (DMSO- d_6) δ 1.40 (s, 9 H), 3.64 (s, 3 H), 3.58 - 3.73 (m, 2 H), 4.32 (bq, $J = 6.2$ Hz, 1 H), 4.49 (s, 2 H), 30 7.16 (bd, $J = 6.2$ Hz, 1 H), 7.25 - 7.38 (m, 5 H); MS (HRFAB, NBA - Li) m/z = 316.1768 $[\text{M} + \text{Li}]^+$; 316.1736 calcd for $\text{C}_{16}\text{H}_{23}\text{O}_5\text{NLi}$.

35 B. Synthesis of DSer(OBzl)-OMe.TFA

-76-

Boc-DSer(OBzl)-OMe (15.0 g, 48.6 mmol) was dissolved in methylene chloride (125 ml) and treated with TFA (32 ml). The resulting mixture was stirred at RT for 30 min and concentrated. The oil was triturated with ether
5 (400 ml) to remove residual TFA to afford 15.9 g (>100 % yield, contains a small amount of residual TFA) of the desired TFA salt as a colorless oil: ¹H NMR (DMSO-d₆) δ 3.72 (s, 3 H), 3.73 - 3.83 (m, 2 H), 4.36 (bt, J = 3.3 Hz, 1 H), 5.50 (ABq, J = 12 Hz, Δ^v = 24 Hz, 2 H), 7.27 -
10 7.37 (m, 5 H), 8.72 (bs, 3 H); MS (HRFAB, NBA - HCl) 210.1159 [M + H]⁺; 210.1130 calcd for C₁₁H₁₆NO₃.

C. Synthesis of Boc-Ser(OBzl)-DSer(OBzl)-OMe

Boc-Ser(OBzl)-OH (14.5 g, 49.2 mmol) was dissolved in
15 DMF (550 ml) and treated with HOBT·H₂O (9.03 g, 59.0 mmol) followed by EDC·HCl (11.3 g, 59.0 mmol). The resulting solution was stirred for 20 min at RT and treated with DSer(OBzl)-OMe·TFA (15.9 g, 49.2 mmol) and TEA (8.22 ml, 59 mmol) and this solution was allowed to
20 stir for 12 h thereafter. The DMF was evaporated and the residue was taken up into ethyl acetate (300 ml). The ethyl acetate solution was washed with 1 N sodium bisulfate (100 ml), water (100 ml), saturated sodium bicarbonate (100 ml), brine (100 ml) and dried (MgSO₄).
25 Filtration and concentration afforded 20.8 g (87 % yield) of the desired dipeptide as a white solid: ¹H NMR (DMSO-d₆) δ 1.37 (s, 9H), 3.57 - 3.75 (m, 4 H), 3.62 (s, 3 H), 4.35 - 4.50 (m, 1 H), 4.46 (s, 3 H), 4.57 - 4.63 (m, 1 H), 6.88 (d, J = 8.1 Hz, 1 H), 7.20 - 7.34
30 (m, 10 H), 8.34 (d, J = 8.1 Hz, 1 H); MS (HRFAB, NBA - Li) m/z = 493.2559 [M + Li]⁺; 493.2526 calcd for C₂₆H₃₄N₂O₇Li.

35 D. Synthesis of Ser(OBzl)-DSer(OBzl)-OMe·TFA

-77-

Boc-Ser(OBzl)-D-Ser(OBzl)-OMe (20.4 g, 42.0 mmol) was dissolved in methylene chloride (170 ml) and TFA (43 ml) was added. The resulting mixture was stirred at RT for 30 min and concentrated. The residue was triturated
5 with ether (400 ml) to remove excess TFA to afford 22.7 g (>100 %, contains excess TFA) of the desired TFA salt:
 ^1H NMR (DMSO- d_6) δ 3.59 - 3.64 (m, 1 H), 3.67 (s, 3 H), 3.70 - 3.85 (m, 3 H), 4.23 (bs, 1 H), 4.40 - 4.58 (m, 4 H), 4.60 - 4.70 (m, 1 H), 7.20 - 7.39 (m, 10 H), 8.41
10 (bs, 3 H), 9.09 (d, J = 7.81 Hz, 1 H); MS (HRFAB, NBA - HCl) m/z = 387.1927 [$M + H$] $^+$; 387.1920 calcd for $C_{21}H_{27}N_2O_5$.

E. Synthesis of Boc-DSer(OBzl)-Ser(OBzl)-DSer(OBzl)-OMe
15 To a solution of Boc-DSer(OBzl)-OH in DMF (480 ml) was added HOBT \cdot H $_2$ O (7.96 g, 52.0 mmol) followed by EDC \cdot HCl (9.97 g, 52.0 mmol) and the resulting solution was stirred for 20 min at RT. To this solution was added Ser(OBzl)-DSer(OBzl)-OMe \cdot TFA (21.7 g, 43.3 mmol) and TEA
20 (7.25 ml, 52.0 mmol) and the resulting mixture was stirred for 16 h thereafter. The DMF was evaporated and the residue was partitioned between water (100 ml) and ethyl acetate (300 ml). The ethyl acetate solution was washed with 1 N sodium bisulfate (150 ml), water (150
25 ml), saturated sodium bicarbonate (150 ml) and brine (150 ml), dried (Na $_2$ SO $_4$), filtered and concentrated to afford 26.6 g (93 % yield) of the desired tripeptide as a white foam: ^1H NMR (DMSO- d_6) δ 1.39 (s, 9 H), 3.56 - 3.74 (complex m, 6 H), 3.64 (s, 3 H), 4.35 - 4.43 (m, 1
30 H), 4.43 - 4.50 (m, 6 H), 4.63 (m, 1 H), 4.73 (m, 1 H), 6.94 (d, J = 7.8 Hz, 1 H), 7.24 - 7.36 (m, 15 H), 8.13 (d, J = 8.2 Hz, 1 H), 8.51 (d, J = 7.8 Hz, 1 H); MS (HRFAB, NBA - Li) m/z = 670.3326 [$M + Li$] $^+$; 670.3316 calcd for $C_{36}H_{45}N_3O_5Li$.

-78-

F. Synthesis of DSer(OBzl)-Ser(OBzl)-DSer(OBzl)-OMe•TFA salt

To a solution of Boc-DSer(OBzl)-Ser(OBzl)-DSer(OBzl)-OMe (26.4 g, 39.7 mmol) in methylene chloride (220 ml) was added TFA (55 ml) and the resulting solution was stirred at RT for 30 min and concentrated. The residue was triturated with ether (300 ml) and the ether triturate was decanted and discarded affording 26.2 g (97 % yield) of the TFA salt as an orange oil after vacuum drying;

¹H NMR (DMSO-d₆) δ 3.55 - 3.3.83 (complex m, 6 H), 3.65 (s, 3 H), 4.21 (bs, 1 H), 4.40 - 4.58 (m, 6 H), 4.60 - 4.67 (m, 1 H), 4.85 - 4.94 (m, 1 H), 7.20 - 7.40 (m, 15 H), 8.37 (bs, 3 H), 8.78 (d, J = 7.8 Hz, 1 H), 8.84 (d, J = 8.3 Hz, 1 H); MS (HRFAB, NBA - Li) m/z = 570.2790 [M + Li]⁺; 570.2792 calcd for C₃₁H₃₇N₃O₇Li.

G. Synthesis of Boc-Ser(OBzl)-DSer(OBzl)-Ser(OBzl)-D-Ser(OBzl)-OMe

To a solution of Boc-Ser(OBzl)-OH (11.2 g, 38.0 mmol) was added HOBT•H₂O (6.99 g, 45.6 mmol) and EDC•HCl (8.74 g, 45.6 mmol) and the resulting mixture was stirred at RT for 30 min. At this time DSer(OBzl)-Ser(OBzl)-DSer(OBzl)-OMe (25.8 g, 38.0 mmol) was added followed by TEA (6.36 ml, 45.6 mmol). The resulting solution was allowed to stir for 16 h thereafter. The DMF was evaporated and the residue was partitioned between water (200 ml) and ethyl acetate (400 ml). The ethyl acetate solution was washed with 1 N sodium bisulfate (200 ml), water (200 ml), saturated sodium bicarbonate (200 ml), and brine (200 ml), dried (MgSO₄), filtered and concentrated to afford 30.2 g (95 % yield) of the desired tetrapeptide as a white foam; ¹H NMR (DMSO-d₆) δ 1.38 (s, 9 H), 3.53 - 3.76 (complex m, 8 H), 3.64 (s, 3 H), 4.34 - 4.42 (m, 1 H), 4.40 - 4.51 (m, 8 H), 4.58 - 4.66 (m, 1 H), 4.67 - 4.77 (m, 1 H), 4.74 - 4.82 (apparent q, J = 7.8 Hz, 1 H), 6.89 (d, J = 8.2 Hz, 1

-79-

H), 7.22 - 7.38 (m, 20 H), 8.14 (d, J = 7.6 Hz, 1 H), 8.34 (d, J = 7.8 Hz, 1 H), 8.55 (d, J = 7.8 Hz, 1 H); MS (HRFAB, NBA - Li) m/z = 847.4095 [M + Li]⁺; 847.4106 calcd for C₄₆H₅₆N₄O₁₁Li.

5

H. Synthesis of Ser(OBzl)-DSer(OBzl)-Ser(OBzl)-DSer(OBzl)-OMe•TFA

Boc-Ser(OBzl)-DSer(OBzl)-Ser(OBzl)-DSer(OBzl)-OMe (30.2 g, 35.9 mmol) was dissolved in methylene chloride (250 ml) and treated with TFA (63 ml). The resulting mixture was stirred for 30 min at RT and concentrated. Trituration with ether afforded 30.0 g (98 % yield) of the desired TFA salt as the oily residue; ¹H NMR (DMSO-d₆) δ 3.50 - 3.85 (complex m, 8 H), 3.65 (s, 3 H), 4.21 (bs, 1 H), 4.40 - 4.55 (m, 8 H), 4.58 - 4.66 (m, 1 H), 4.80 - 4.95 (m, 2 H), 7.20 - 7.40 (m, 20 H), 8.35 (bs, 3 H), 8.54 (d, J = 8.2 Hz, 1 H), 8.69 (d, J = 7.4 Hz, 1 H), 8.86 (d, J = 8.2 Hz, 1 H); MS (HRFAB, NBA - Li) m/z = 747.3590 [M + Li]⁺; 747.3581 calcd for C₄₁H₄₈N₄O₉Li.

20

I. Synthesis of Boc-Ser(OBzl)-Ser(OBzl)-DSer(OBzl)-Ser(OBzl)-DSer(OBzl)-OMe

To a solution of Boc-Ser(OBzl)-OH (10.1 g, 34.2 mmol) in DMF (380 ml) was added HOBT•H₂O (6.28 g, 41.0 mmol) followed by EDC•HCl (7.86 g, 41.0 mmol) and the resulting mixture was stirred for 20 min at RT. Ser(OBzl)-DSer(OBzl)-Ser(OBzl)-DSer(OBzl)-OMe•TFA (29.2 g, 34.2 mmol) was added followed by the addition of TEA (5.71 ml, 41.0 mmol) and the reaction was allowed to stir for 12 h thereafter. The DMF was evaporated and the residue was partitioned between water (200 ml) and ethyl acetate (400 ml). The ethyl acetate layer was washed with 1 N sodium bisulfate (200 ml), water (200 ml), saturated sodium bicarbonate (200 ml) and brine (200 ml), dried (MgSO₄), filtered and concentrated to

-80-

afford 33.7 g (97 % yield) of the desired pentapeptide as a white powder; ¹H NMR (DMSO-d₆) δ 1.39 (s, 9 H), 3.55 - 3.77 (complex m, 10 H), 3.64 (s, 3 H), 4.32 - 4.42 (m, 1 H), 4.40 - 4.54 (m, 10 H), 4.60 - 4.68 (m, 1 H), 4.49 - 4.84 (m, 3 H), 7.00 (d, J = 7.7 Hz, 1 H), 7.20 - 7.40 (m, 25 H), 8.11 (d, J = 7.4 Hz, 1 H), 8.30 (d, J = 7.8 Hz, 1 H), 8.35 (d, J = 7.8 Hz, 1 H), 8.58 (d, J = 7.8 Hz, 1 H); MS (LRFAB, NBA - Li) m/z = 1024 [M + Li]⁺.

10

J. Synthesis Ser(OBzl)-Ser(OBzl)-DSer(OBzl)-Ser(OBzl)-DSer(OBzl)•HCl

Boc-Ser(OBzl)-Ser(OBzl)-DSer(OBzl)-Ser(OBzl)-DSer(OBzl)-OMe (31.7 g, 31.1 mmol) was dissolved in acetic acid (760 ml) and treated with concentrated HCl (190 ml). The resulting solution was stirred at RT for 12 h thereafter. Concentration afforded 29.3 g (100 %) of the desired deprotected peptide salt as a white foam; ¹H NMR (DMSO-d₆) δ 3.55 - 3.90 (complex m, 10 H), 4.20 (m, 1 H), 4.38 - 4.61 (m, 11 H), 4.73 - 4.85 (m, 3 H), 7.20 - 7.40 (m, 25 H), 8.34 - 8.42 (m, 2 H), 8.49 (m, 4 H), 8.95 (d, J = 7.7 Hz, 1 H); (LRFAB, NBA - HCl) m/z = 905 [M + H]⁺.

25 K. Synthesis of Cyclo-[Ser(OBzl)-Ser(OBzl)-DSer(OBzl)-Ser(OBzl)-DSer(OBzl)-]

A solution of Ser(OBzl)-Ser(OBzl)-DSer(OBzl)-Ser(OBzl)-DSer(OBzl)•HCl (29.4 g, 31.3 mmol) in anhydrous degassed DMF (4000 ml) was cooled to -40 °C and treated with DPPA (8.22 ml, 38.1 mmol) followed by TEA (4.53 ml, 32.5 mmol). The reaction was stored at -20 °C for 48 h and at 0 °C for 48 h thereafter. During this time the "pH" was maintained at -8 by periodic addition of TEA (measured by spotting reaction mixture on moistened Hydrion paper). After this reaction period the reaction was diluted with water (1000 ml) and stirred with mixed

-81-

bed ion-exchange resin (1600 g) for 6 h. The resin was removed by filtration and the filtrate was concentrated to a volume of ~200 ml. The product was precipitated by the addition of water (500 ml). The solid was filtered
 5 and washed with ether (250 ml) to afford after vacuum desiccation 20.5 g (74 % yield) of the desired cyclic peptide as a white powder: ¹H NMR (DMSO-d₆, major conformer) δ 3.50 - 3.83 (complex m, 11 H), 4.32 - 4.82 (complex m, 14 H), 7.18 - 7.38 (m, 25 H), 7.56 (d, J =
 10 6.8 Hz, 1 H), 7.94 (d, J = 8.7 Hz, 1 H), 8.50 (d, J = 6.0 Hz, 1 H), 8.78 (m, 2 H); ; (LRFAB, NBA - Li) m/z = 893 [M + Li]⁺.

L. Synthesis of (2S, 5R, 8S, 11R, 14S)-

15 Penta(benzyloxymethyl)-1,4, 7, 10, 13-pentaazacyclo-pentadecane.

To a solution of the Cyclo-[Ser(OBzl)-Ser(OBzl)-DSer(OBzl)-Ser(OBzl)-DSer(OBzl)-] (7.00 g, 7.90 mmol) in THF (100 ml) was added lithium aluminum hydride (100 ml
 20 of a 1.0 M solution in THF, 100 mmol) over 5 min at RT. The resulting solution was heated at reflux for 16 h thereafter. The mixture was cooled to --20 °C and quenched (cautiously) with 5% sodium sulfate solution (30 ml). The resulting mixture was concentrated to a
 25 white powder and this powder was thoroughly dried by coevaporation with toluene (3 x 50 ml). The residue was triturated with ether (2 x 100 ml) and the combined triturations were concentrated to afford 6.1 g (95 %
 30 yield) of the desired ligand as a yellow oil; ¹H NMR (CDCl₃) δ 1.95 (bs, 5 H), 2.30 - 3.60 (complex m, 25 H), 4.20 - 4.40 (m, 10 H), 7.00 - 7.38 (m, 25 H); MS (HRFAB, NBA - Li) m/z = 822.5174 [M + Li]⁺; 822.5146 calcd for C₃₀H₄₃N₅O₅Li.

-82-

M. Synthesis of [Manganese(II)dichloro(2S, 5R, 8S, 11R, 14S)-penta(hydroxymethyl)-1,4,7, 10,13-pentaazacyclopentadecane.

To a solution of manganese(II) chloride (221 mg, 1.76 mmol) in methanol (75 ml) was added (2S, 5R, 8S, 11R, 14S)-penta(benzyloxymethyl)-1,4,7,10,13-pentaazacyclopentadecane (1.43 g, 1.75 mmol) and the reaction mixture was refluxed for 2 h and stirred at RT for 12 h thereafter. The methanol solution was evaporated and the residual oil was redissolved in ethanol (10 ml) and water (1 ml) in a Parr bottle. This mixture was treated with 10 % Pd on carbon (1 g) and hydrogenolyzed at 60 °C and 65 psi for 16 h thereafter. Filtration, concentration and recrystallization from ethanol-ether afforded 212 mg (29 % yield) of the desired complex as a white solid; MS (HRFAB, NBA) m/z = 455.1718 [M - Cl]⁺; 455.1707 calcd for C₁₅H₃₃N₅MnCl.

-83-

Example 6

Relaxivity measurements of the complexes of the invention (Examples 2-5) and of a comparative complex (Example 1) were determined and the results are found in Table I.

Proton relaxation times (T_1) of the sample in 100mM Hepes buffer, pH=7.4, at 40°C were determined from a monoexponential curve fit obtained from inversion-recovery pulse sequences (180° - τ - 90°) with a Bruker PC 120/125/10 VTs NMR process analyzer. The spectrometer was calibrated for each sample to assure accurate duration of 90° and 180° radio frequency pulses and appropriate magnetic field strength to match the 20 MHz system operating frequency. The relaxivity (R_1) was obtained from the slope of a plot of $1/T_1$ versus the concentration of paramagnetic compound.

The relaxation time (T_2) of each sample in 100 mM Hepes buffer, pH=7.4, was measured at 40°C using a Carr-Purcel-Meiboom-Gill pulse sequence on the same Bruker instrument. The relaxivity (R_2) was obtained from a plot of $1/T_2$ versus the concentration of the paramagnetic compound.

25

Table I

	<u>Sample</u>	Relaxivity, mM ⁻¹ sec ⁻¹	
		R_1	R_2
	Example 1 (Comparative)	1.82	2.37
30	Example 2	2.09	2.49
	Example 3	2.76	3.86
	Example 4	2.10	2.61
	Example 5	3.48	5.93

35

-84-

The results found in Table I demonstrate that the complexes of the invention (Examples 2-5) have improved relaxivities compared to the comparative complex (Example 1).

5

Example 7

Kinetic stability measurements of the complexes of the invention (Examples 2-4) and of the comparative complex (Example 1) were determined and the results are found in Table II.

The kinetic stability constant ($k_{d,111}$) for each complex can be determined by observing the rate at which copper appears to replace manganese as the metal center of the manganese macrocyclic complexes at different pH's.

A Beckman model DU-70 UV/VIS spectrometer was set up to scan the wavelengths from 700 to 200 nanometers. Concentrations of the complexes were kept low enough to stay within the linear range of observable absorbents for the DU-70, which is below 2.00 absorbents units.

Since copper has an observable absorbency band within the experimental range of wavelengths and binding energies that are much higher than that of manganese, the replacement of copper for manganese as the metal center is almost instantaneous. Therefore, copper is a good choice as a tracer of the decomposition of manganese from the macrocyclic ligand system.

30

35

-85-

Table II

<u>Example No.</u>	Kinetic Stability, <u>k_{obs} ($\text{M}^{-1}\text{Sec}^{-1}$)</u>
5 1 (Comparative)	2814
2	1375
3	17.7
4	31.5
5	ND ¹
10 ¹ Not Determined	

The data in Table II demonstrates that the complexes of the invention (Examples 2-4) are substantially more kinetically stable than the comparative complex (Example 1).

Example 8

Oxidation potentials of the complexes of the invention (Examples 2-4) and of the comparative complex (Example 1) were determined and the results are found in Table III.

Cyclic voltammograms were run in methanol containing 0.18M tetrabutylammonium chloride under nitrogen using a glassy carbon electrode with a platinum reference electrode and ferrocene internal standard.

Table III

<u>Example No.</u>	Oxidation Potential, <u>$E_{1/2}$ (volts)</u>
1 (Comparative)	0.70
2	0.75
3	0.85
4	0.74
5	ND ¹
¹ Not Determined	

-86-

The results in Table III demonstrates that the manganese(II) complexes of the invention (Examples 2-4) are more oxidatively stable than the comparative manganese(II) complex (Example 1).

5

Example 9

The partition coefficient (log P) of the complexes of the invention (Examples 2-5) and of a comparative complex of (Example 1) were determined and the results are found in Table IV.

The partition coefficient was determined by measuring the manganese contents in each of the buffer and octanol phases. About 5mg of sample was dissolved in a 5mL octanol saturated buffer solution (10 mM Hepes/150mM NaCl, pH=7.4). The sample solution was mixed with 5mL of buffer saturated octanol and shaken overnight. The mixed solution was centrifuged to separate the two phases. 2mL of solution (in duplicate) was taken from each phase and digested with an acid mixture. The prepared solutions were then measured for manganese using ICP-AES and/or ICP-MS. The log P value was calculated from the Mn(octanol)/Mn(buffer) ratio.

25

Table IV

Example No.	Partition Coefficient, log P
1 (Comparative)	-2.9
2	-1.9
3	-1.1
4	-0.76
5	-3.2

35

The results in Table IV demonstrate that the log P, i.e. lipophilicity or biodistribution, of the complexes

-87-

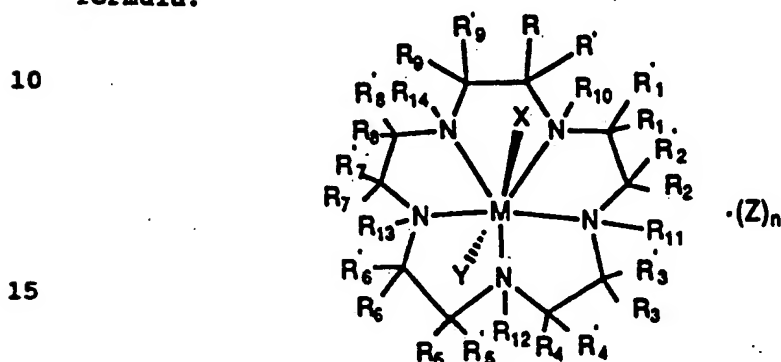
of the invention (Examples 2-5) can be controlled by controlling the substituents on the macrocycle.

CLAIMS

That which is claimed is:

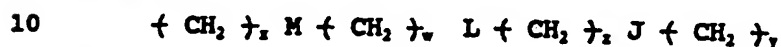
1. A method of magnetic resonance imaging comprising:

- 5 (a) administering to a human or non-human animal subject a contrast medium comprising a physiologically compatible complex represented by the formula:



wherein R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉, and R'₉, independently are
 20 selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, cycloalkylcycloalkyl, cycloalkenylalkyl, alkylcycloalkyl, alkenylcycloalkyl, alkylcycloalkenyl, alkenylcycloalkenyl, heterocyclic,
 25 aryl and aralkyl radicals and radicals attached to the α-carbon of α-amino acids; or R₁ or R'₁ and R₂ or R'₂, R₃ or R'₃, and R₄ or R'₄, R₅ or R'₅, and R₆ or R'₆, R₇ or R'₇, and R₈ or R'₈, and R₉ or R'₉, and R or R' together with the carbon atoms to which they are attached independently
 30 form a saturated, partially saturated or unsaturated cyclic having 3 to 20 carbon atoms; R and R', R₁ and R'₁, R₂ and R'₂, R₃ and R'₃, R₄ and R'₄, R₅ and R'₅, R₆ and R'₆, R₇ and R'₇, R₈ and R'₈, and R₉ and R'₉, together with the carbon atoms to which they are attached
 35 independently form a saturated, partially saturated, or

unsaturated ring structure having 3 to 20 carbon atoms; or one of R , R' , R_1 , R'_1 , R_2 , R'_2 , R_3 , R'_3 , R_4 , R'_4 , R_5 , R'_5 , R_6 , R'_6 , R_7 , R'_7 , R_8 , R'_8 , R_9 , R'_9 , R_{10} , R_{11} , R_{12} , R_{13} and R_{14} together with a different one of R , R' , R_1 , R'_1 , R_2 , R'_2 , R_3 , R'_3 , R_4 , R'_4 , R_5 , R'_5 , R_6 , R'_6 , R_7 , R'_7 , R_8 , R'_8 , R_9 , R'_9 , R_{10} , R_{11} , R_{12} , R_{13} and R_{14} which is attached to a different carbon or nitrogen atom in the macrocyclic ligand may be bound to form a strap represented by the formula



wherein w , x , y and z independently are integers from 0 to 10, and M , L and J are independently selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, cycloalkyl, heteroaryl, alkaryl, alkheteroaryl, aza, amide, ammonium, thia, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphino, phosphonium, keto, ester, carbamate, urea, thiocarbonyl, borates, boranes, boraza, silyl, siloxy, silaza and combinations thereof; and R_{10} , R_{11} , R_{12} , R_{13} and R_{14} independently are selected from the group consisting of hydrogen, alkyl, and alkyl substituted with $-\text{OR}_{15}$, $-\text{COOR}_{15}$, $-\text{CONR}_{15}\text{R}_{16}$ or $-\text{PO}_3\text{H}_2$, wherein R_{15} and R_{16} are independently hydrogen or alkyl; wherein at least two of R , R' , R_1 , R'_1 , R_2 , R'_2 , R_3 , R'_3 , R_4 , R'_4 , R_5 , R'_5 , R_6 , R'_6 , R_7 , R'_7 , R_8 , R'_8 , R_9 and R'_9 are other than hydrogen; wherein X , Y and Z are ligands independently selected from the group consisting of halide, oxo, aquo, hydroxo, alcohol, phenol, dioxygen, peroxy, hydroperoxy, alkylperoxy, arylperoxy, ammonia, alkylamino, arylamino, heterocycloalkyl amino, heterocycloaryl amino, amine oxides, hydrazine, alkyl hydrazine, aryl hydrazine, nitric oxide, cyanide, cyanate, thiocyanate, isocyanate, isothiocyanate, alkyl nitrile, aryl nitrile, alkyl isonitrile, aryl isonitrile, nitrate, nitrite, azido, alkyl sulfonic acid, aryl sulfonic acid, alkyl sulfoxide, aryl

-90-

- sulfoxide, alkyl aryl sulfoxide, alkyl sulfenic acid, aryl sulfenic acid, alkyl sulfinic acid, aryl sulfinic acid, alkyl thiol carboxylic acid, aryl thiol carboxylic acid, alkyl thiol thiocarboxylic acid, aryl thiol thiocarboxylic acid, alkyl carboxylic acid, aryl carboxylic acid, urea, alkyl urea, aryl urea, alkyl aryl urea, thiourea, alkyl thiourea, aryl thiourea, alkyl aryl thiourea, sulfate, sulfite, bisulfate, bisulfite, thiosulfate, thiosulfite, hydrosulfite, alkyl phosphine, aryl phosphine, alkyl phosphine oxide, aryl phosphine oxide, alkyl aryl phosphine oxide, alkyl phosphine sulfide, aryl phosphine sulfide, alkyl aryl phosphine sulfide, alkyl phosphonic acid, aryl phosphonic acid, alkyl phosphinic acid, aryl phosphinic acid, alkyl phosphinous acid, aryl phosphinous acid, phosphate, thiophosphate, phosphite, pyrophosphite, triphosphate, hydrogen phosphate, dihydrogen phosphate, alkyl guanidino, aryl guanidino, alkyl aryl guanidino, alkyl carbamate, aryl carbamate, alkyl aryl carbamate, alkyl thiocarbamate, aryl thiocarbamate, alkylaryl thiocarbamate, alkyl dithiocarbamate, aryl dithiocarbamate, alkylaryl dithiocarbamate, bicarbonate, carbonate, perchlorate, chlorate, chlorite, hypochlorite, perbromate, bromate, bromite, hypobromite, tetrahalomanganate, tetrafluoroborate, hexafluoroantimonate, hypophosphite, iodate, periodate, metaborate, tetraaryl borate, tetra alkyl borate, tartrate, salicylate, succinate, citrate, ascorbate, saccharinate, amino acid, hydroxamic acid, thiotosylate, and anions of ion exchange resins, or the corresponding anions thereof, or X, Y and Z are independently attached to one or more of the "R" groups and n is an integer from 0 to 3;
- M is a paramagnetic metal selected from the group consisting of metals having atomic numbers 21-29, 42-44

-91-

and 57-71; and a nontoxic, pharmaceutically acceptable carrier, adjuvant or vehicle; and

(b) generating a magnetic resonance image of at least a part of said subject.

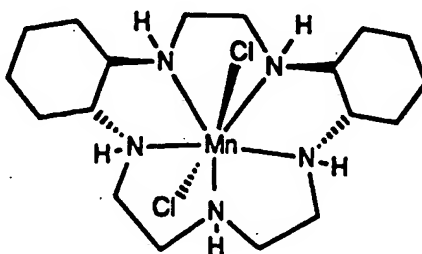
5 2. The method of Claim 1 wherein M is Mn(II) or Gd(III).

3. The method of Claim 2 wherein M is Mn(II).

4. The method of Claim 1 wherein at least three of R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆,
10 R₇, R'₇, R₈, R'₈, R₉ and R'₉, are other than hydrogen.

5. The method of Claim 1 wherein at least one of R₁ or R'₁, and R₂ or R'₂, R₃ or R'₃, and R₄ or R'₄, R₅ or R'₅, and R₆ or R'₆, R₇ or R'₇, and R₈ or R'₈, and R₉ or R'₉, and R or R' together with the carbon atoms to which they are
15 attached form a saturated cyclic having 5 to 8 carbon atoms; and all of the remaining "R" groups are independently selected from hydrogen, alkyl, or alkyl substituted with -OR₁₅ or -NR₁₅R₁₆, wherein R₁₅ and R₁₆ are independently hydrogen or alkyl.

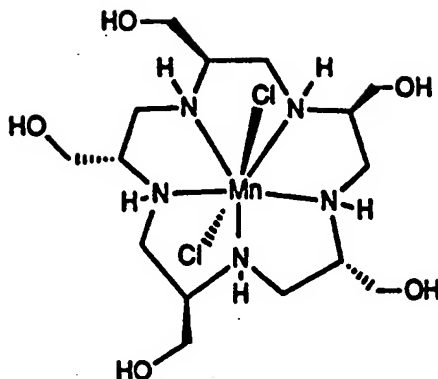
20 6. The method of Claim 5 wherein said complex is represented by the formula:



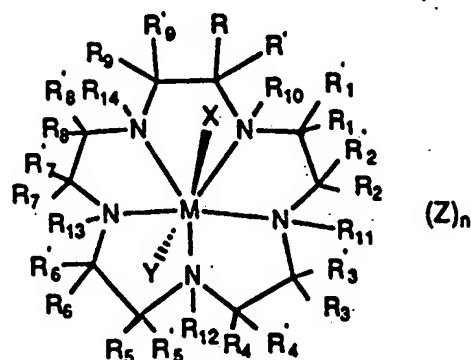
25 7. The method of Claim 1 wherein at least two of R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆,
30 R₇, R'₇, R₈, R'₈, R₉ and R'₉, are alkyl or alkyl substituted with -OR₁₅ or -NR₁₅R₁₆, wherein R₁₅ and R₁₆ are independently hydrogen or alkyl.

8. The method of Claim 7 wherein said complex is
35 represented by the formula:

-92-

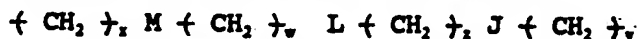


9. A method of diagnostic imaging comprising:
- (a) administering to a human or non-human animal
 subject a diagnostic agent comprising a physiologically
 15 compatible complex represented by the formula:



wherein R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅,
 R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉, and R', independently are
 selected from the group consisting of hydrogen, alkyl,
 alkenyl, alkynyl, cycloalkyl, cycloalkenyl,
 30 cycloalkylalkyl, cycloalkylcycloalkyl,
 cycloalkenylalkyl, alkylcycloalkyl, alkenylcycloalkyl,
 alkylcycloalkenyl, alkenylcycloalkenyl, heterocyclic,
 aryl and aralkyl radicals and radicals attached to the
 α-carbon of α-amino acids; or R₁ or R'₁ and R₂ or R'₂, R₃
 35 or R'₃, and R₄ or R'₄, R₅ or R'₅, and R₆ or R'₆, R₇ or R'₇, and
 R₈ or R'₈, and R₉ or R', and R or R' together with the

carbon atoms to which they are attached independently form a saturated, partially saturated or unsaturated cyclic having 3 to 20 carbon atoms; R and R', R₁ and R'₁, R₂ and R'₂, R₃ and R'₃, R₄ and R'₄, R₅ and R'₅, R₆ and R'₆,
 5 R₇ and R'₇, R₈ and R'₈, and R₉ and R'₉, together with the carbon atoms to which they are attached independently form a saturated, partially saturated, or unsaturated ring structure having 3 to 20 carbon atoms; or one of R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇,
 10 R'₇, R₈, R'₈, R₉, R'₉, R₁₀, R₁₁, R₁₂, R₁₃ and R₁₄ together with a different one of R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉, R'₉, R₁₀, R₁₁, R₁₂, R₁₃ and R₁₄ which is attached to a different carbon or nitrogen atom in the macrocyclic ligand may be bound to
 15 form a strap represented by the formula



wherein w, x, y and z independently are integers from 0 to 10, and M, L and J are independently selected from the group consisting of alkyl, alkenyl, alkynyl, aryl,
 20 cycloalkyl, heteroaryl, alkaryl, alkheteroaryl, aza, amide, ammonium, thia, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphino, phosphonium, keto, ester, carbamate, urea, thiocarbonyl, borates, boranes, boraza, silyl, siloxy, silaza and combinations thereof;
 25 and R₁₀, R₁₁, R₁₂, R₁₃ and R₁₄ independently are selected from the group consisting of hydrogen, alkyl, and alkyl substituted with -OR₁₅, -COOR₁₅, -CONR₁₅R₁₆ or -PO₃H₂, wherein R₁₅ and R₁₆ are independently hydrogen or alkyl; wherein at least two of R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃,
 30 R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉ and R'₉ are other than hydrogen; wherein X, Y and Z are ligands independently selected from the group consisting of halide, oxo, aquo, hydroxo, alcohol, phenol, dioxygen, peroxy, hydroperoxy, alkylperoxy, arylperoxy, ammonia,
 35 alkylamino, arylamino, heterocycloalkyl amino,

heterocycloaryl amino, amine oxides, hydrazine, alkyl hydrazine, aryl hydrazine, nitric oxide, cyanide, cyanate, thiocyanate, isocyanate, isothiocyanate, alkyl nitrile, aryl nitrile, alkyl isonitrile, aryl isonitrile, nitrate, nitrite, azido, alkyl sulfonic acid, aryl sulfonic acid, alkyl sulfoxide, aryl sulfoxide, alkyl aryl sulfoxide, alkyl sulfenic acid, aryl sulfenic acid, alkyl sulfinic acid, aryl sulfinic acid, alkyl thiol carboxylic acid, aryl thiol carboxylic acid, alkyl thiol thiocarboxylic acid, aryl thiol thiocarboxylic acid, alkyl carboxylic acid, aryl carboxylic acid, urea, alkyl urea, aryl urea, alkyl aryl urea, thiourea, alkyl thiourea, aryl thiourea, alkyl aryl thiourea, sulfate, sulfite, bisulfate, bisulfite, thiosulfate, thiosulfite, hydrosulfite, alkyl phosphine, aryl phosphine, alkyl phosphine oxide, aryl phosphine oxide, alkyl aryl phosphine oxide, alkyl phosphine sulfide, aryl phosphine sulfide, alkyl aryl phosphine sulfide, alkyl phosphonic acid, aryl phosphonic acid, alkyl phosphinic acid, aryl phosphinic acid, alkyl phosphinous acid, aryl phosphinous acid, phosphate, thiophosphate, phosphite, pyrophosphite, triphosphate, hydrogen phosphate, dihydrogen phosphate, alkyl guanidino, aryl guanidino, alkyl aryl guanidino, alkyl carbamate, aryl carbamate, alkyl aryl carbamate, alkyl thiocarbamate, aryl thiocarbamate, alkylaryl thiocarbamate, alkyl dithiocarbamate, aryl dithiocarbamate, alkylaryl dithiocarbamate, bicarbonate, carbonate, perchlorate, chlorate, chlorite, hypochlorite, perbromate, bromate, bromite, hypobromite, tetrahalomanganate, tetrafluoroborate, hexafluoroantimonate, hypophosphite, iodate, periodate, metaborate, tetraaryl borate, tetra alkyl borate, tartrate, salicylate, succinate, citrate, ascorbate, saccharinate, amino acid, hydroxamic acid, thiotosylate, and anions of ion exchange resins, or the corresponding

-95-

anions thereof, or X, Y and Z are independently attached to one or more of the "R" groups and n is an integer from 0 to 3;

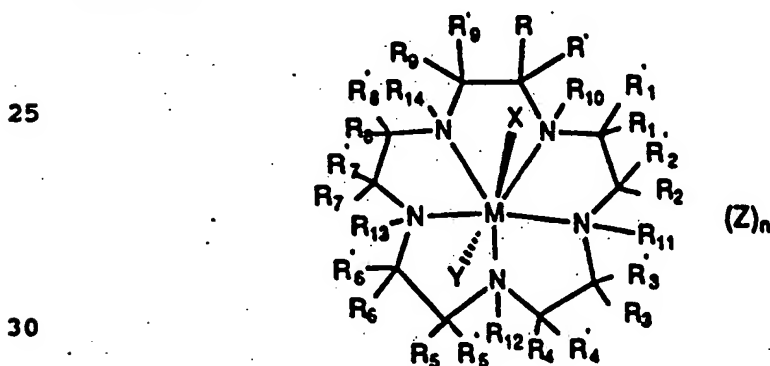
M is a heavy metal selected from the group consisting of metals having atomic numbers 20-32, 42-44, 49 and 57-83; and a nontoxic, pharmaceutically acceptable carrier, adjuvant or vehicle; and

(b) generating an X-ray, ultrasound or scintigraphic image of at least a part of said subject.

10 10. The method of Claim 9 wherein M is a radioactive metal isotope selected from the group consisting of ^{99m}Tc and ^{111}In and said image is a scintigraphic image.

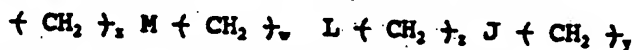
11. The method of Claim 9 wherein at least three
15 of R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉, and R'₉, are other than hydrogen.

12. A method of radiotherapy practiced on a human or non-human animal subject comprising administering to
20 said subject a radioactive agent comprising a physiologically compatible complex represented by the formula:



wherein R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉, and R'₉, independently are selected from the group consisting of hydrogen, alkyl,
35 alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, cycloalkylcycloalkyl,

cycloalkenylalkyl, alkylcycloalkyl, alkenylcycloalkyl, alkylcycloalkenyl, alkenylcycloalkenyl, heterocyclic, aryl and aralkyl radicals and radicals attached to the α -carbon of α -amino acids; or R_1 or R'_1 , and R_2 or R'_2 , R_3 or R'_3 , and R_4 or R'_4 , R_5 or R'_5 , and R_6 or R'_6 , R_7 or R'_7 , and R_8 or R'_8 , and R_9 or R'_9 , and R or R' together with the carbon atoms to which they are attached independently form a saturated, partially saturated or unsaturated cyclic having 3 to 20 carbon atoms; R and R' , R_1 and R'_1 , R_2 and R'_2 , R_3 and R'_3 , R_4 and R'_4 , R_5 and R'_5 , R_6 and R'_6 , R_7 and R'_7 , R_8 and R'_8 , and R_9 and R'_9 , together with the carbon atoms to which they are attached independently form a saturated, partially saturated, or unsaturated ring structure having 3 to 20 carbon atoms; or one of R , R' , R_1 , R'_1 , R_2 , R'_2 , R_3 , R'_3 , R_4 , R'_4 , R_5 , R'_5 , R_6 , R'_6 , R_7 , R'_7 , R_8 , R'_8 , R_9 , R'_9 , R_{10} , R_{11} , R_{12} , R_{13} , and R_{14} together with a different one of R , R' , R_1 , R'_1 , R_2 , R'_2 , R_3 , R'_3 , R_4 , R'_4 , R_5 , R'_5 , R_6 , R'_6 , R_7 , R'_7 , R_8 , R'_8 , R_9 , R'_9 , R_{10} , R_{11} , R_{12} , R_{13} , and R_{14} which is attached to a different carbon or nitrogen atom in the macrocyclic ligand may be bound to form a strap represented by the formula



wherein w , x , y and z independently are integers from 0 to 10, and M , L and J are independently selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, cycloalkyl, heteroaryl, alkaryl, alkheteroaryl, aza, amide, ammonium, thia, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphino, phosphonium, keto, ester, carbamate, urea, thiocarbonyl, borates, boranes, boraza, silyl, siloxy, silaza and combinations thereof; and R_{10} , R_{11} , R_{12} , R_{13} , and R_{14} independently are selected from the group consisting of hydrogen, alkyl, and alkyl substituted with $-\text{OR}_{15}$, $-\text{COOR}_{15}$, $-\text{CONR}_{15}\text{R}_{16}$ or $-\text{PO}_3\text{H}_2$ wherein R_{15} and R_{16} are independently hydrogen or alkyl; wherein at least two of R , R' , R_1 , R'_1 , R_2 , R'_2 , R_3 , R'_3 ,

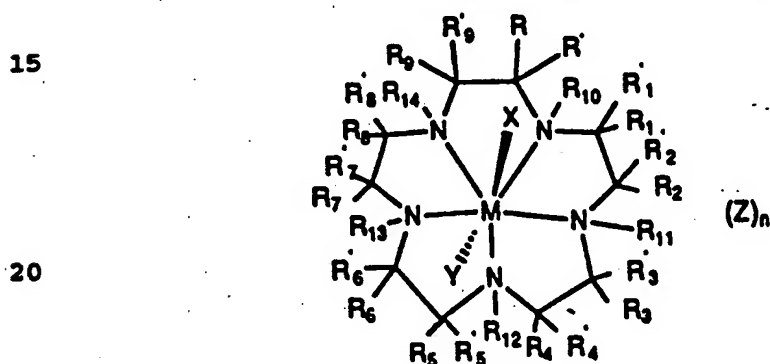
-97-

R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉ and R', are other than hydrogen; wherein X, Y and Z are ligands independently selected from the group consisting of halide, oxo, aquo, hydroxo, alcohol, phenol, dioxygen, peroxy, hydroperoxy, alkylperoxy, arylperoxy, ammonia, 5 alkylamino, arylamino, heterocycloalkyl amino, heterocycloaryl amino, amine oxides, hydrazine, alkyl hydrazine, aryl hydrazine, nitric oxide, cyanide, cyanate, thiocyanate, isocyanate, isothiocyanate, alkyl 10 nitrile, aryl nitrile, alkyl isonitrile, aryl isonitrile, nitrate, nitrite, azido, alkyl sulfonic acid, aryl sulfonic acid, alkyl sulfoxide, aryl sulfoxide, alkyl aryl sulfoxide, alkyl sulfenic acid, aryl sulfenic acid, alkyl sulfinic acid, aryl sulfinic 15 acid, alkyl thiol carboxylic acid, aryl thiol carboxylic acid, alkyl thiol thiocarboxylic acid, aryl thiol thiocarboxylic acid, alkyl carboxylic acid, aryl carboxylic acid, urea, alkyl urea, aryl urea, alkyl aryl urea, thiourea, alkyl thiourea, aryl thiourea, alkyl 20 aryl thiourea, sulfate, sulfite, bisulfate, bisulfite, thiosulfate, thiosulfite, hydrosulfite, alkyl phosphine, aryl phosphine, alkyl phosphine oxide, aryl phosphine oxide, alkyl aryl phosphine oxide, alkyl phosphine sulfide, aryl phosphine sulfide, alkyl aryl phosphine sulfide, alkyl phosphonic acid, aryl phosphonic acid, 25 alkyl phosphinic acid, aryl phosphinic acid, alkyl phosphinous acid, aryl phosphinous acid, phosphate, thiophosphate, phosphite, pyrophosphite, triphosphate, hydrogen phosphate, dihydrogen phosphate, alkyl 30 guanidino, aryl guanidino, alkyl aryl guanidino, alkyl carbamate, aryl carbamate, alkyl aryl carbamate, alkyl thiocarbamate, aryl thiocarbamate, alkylaryl thiocarbamate, alkyl dithiocarbamate, aryl dithiocarbamate, alkylaryl dithiocarbamate, bicarbonate, 35 carbonate, perchlorate, chlorate, chlorite, hypochlorite, perbromate, bromate, bromite, hypobromite,

-98-

- tetrahalomanganate, tetrafluoroborate, hexafluoroantimonate, hypophosphite, iodate, periodate, metaborate, tetraaryl borate, tetra alkyl borate, tartrate, salicylate, succinate, citrate, ascorbate, saccharinate, amino acid, hydroxamic acid, thiotosylate, and anions of ion exchange resins, or the corresponding anions thereof, or X, Y and Z are independently attached to one or more of the "R" groups and n is an integer from 0 to 3;
- 10 M is a radioactive metal selected from ^{153}Sm , ^{67}Cu or ^{90}Y ; and a nontoxic, pharmaceutically acceptable carrier, adjuvant or vehicle.

13. A compound represented by the formula:



- wherein R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉, and R', independently are
- 25 selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, cycloalkylcycloalkyl, cycloalkenylalkyl, alkylcycloalkyl, alkenylcycloalkyl, alkylcycloalkenyl, alkenylcycloalkenyl, heterocyclic,
- 30 aryl and aralkyl radicals and radicals attached to the α -carbon of α -amino acids; or R₁ or R'₁ and R₂ or R'₂, R₃ or R'₃ and R₄ or R'₄, R₅ or R'₅ and R₆ or R'₆, R₇ or R'₇ and R₈ or R'₈, and R₉ or R', and R or R' together with the carbon atoms to which they are attached independently
- 35 form a saturated, partially saturated or unsaturated

-99-

cyclic having 3 to 20 carbon atoms; R and R', R₁ and R'₁, R₂ and R'₂, R₃ and R'₃, R₄ and R'₄, R₅ and R'₅, R₆ and R'₆, R₇ and R'₇, R₈ and R'₈, and R₉ and R', together with the carbon atoms to which they are attached

- 5 independently form a saturated, partially saturated, or unsaturated ring structure having 3 to 20 carbon atoms; or one of R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉, R'₉, R₁₀, R₁₁, R₁₂, R₁₃ and R₁₄ together with a different one of R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉, R'₉, R₁₀, R₁₁, R₁₂, R₁₃ and R₁₄ which is attached to a different carbon or nitrogen atom in the macrocyclic ligand may be bound to form a strap represented by the formula

- 15 $\text{-(CH}_2\text{)}_w\text{-M-(CH}_2\text{)}_x\text{-L-(CH}_2\text{)}_y\text{-J-(CH}_2\text{)}_z\text{-}$
 wherein w, x, y and z independently are integers from 0 to 10, and M, L and J are independently selected from the group consisting of alkyl, alkenyl, alkynyl, aryl, cycloalkyl, heteroaryl, alkaryl, alkheteroaryl, aza, amide, ammonium, thia, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, phosphino, phosphonium, keto, ester, carbamate, urea, thiocarbonyl, borates, boranes, boraza, silyl, siloxy, silaza and combinations thereof; and R₁₀, R₁₁, R₁₂, R₁₃ and R₁₄ independently are selected from the group consisting of hydrogen, alkyl, and alkyl substituted with -OR₁₅, -COOR₁₅, -CONR₁₅R₁₆ or -PO₃H₂ wherein R₁₅ and R₁₆ are independently hydrogen or alkyl; wherein at least two of R, R', R₁, R'₁, R₂, R'₂, R₃, R'₃, R₄, R'₄, R₅, R'₅, R₆, R'₆, R₇, R'₇, R₈, R'₈, R₉ and R' are other than hydrogen; wherein X, Y and Z are ligands independently selected from the group consisting of halide, oxo, aquo, hydroxo, alcohol, phenol, dioxygen, peroxo, hydroperoxo, alkylperoxo, arylperoxo, ammonia, alkylamino, arylamino, heterocycloalkyl amino, heterocycloaryl amino, amine

-100-

- oxides, hydrazine, alkyl hydrazine, aryl hydrazine, nitric oxide, cyanide, cyanate, thiocyanate, isocyanate, isothiocyanate, alkyl nitrile, aryl nitrile, alkyl isonitrile, aryl isonitrile, nitrate, nitrite, azido,
- 5 alkyl sulfonic acid, aryl sulfonic acid, alkyl sulfoxide, aryl sulfoxide, alkyl aryl sulfoxide, alkyl sulfenic acid, aryl sulfenic acid, alkyl sulfinic acid, aryl sulfinic acid, alkyl thiol carboxylic acid, aryl thiol carboxylic acid, alkyl thiol thiocarboxylic acid,
- 10 aryl thiol thiocarboxylic acid, alkyl carboxylic acid, aryl carboxylic acid, urea, alkyl urea, aryl urea, alkyl aryl urea, thiourea, alkyl thiourea, aryl thiourea, alkyl aryl thiourea, sulfate, sulfite, bisulfate, bisulfite, thiosulfate, thiosulfite, hydrosulfite, alkyl
- 15 phosphine, aryl phosphine, alkyl phosphine oxide, aryl phosphine oxide, alkyl aryl phosphine oxide, alkyl phosphine sulfide, aryl phosphine sulfide, alkyl aryl phosphine sulfide, alkyl phosphonic acid, aryl phosphonic acid, alkyl phosphinic acid, aryl phosphinic acid, alkyl phosphinous acid, aryl phosphinous acid,
- 20 phosphate, thiophosphate, phosphite, pyrophosphite, triphosphate, hydrogen phosphate, dihydrogen phosphate, alkyl guanidino, aryl guanidino, alkyl aryl guanidino, alkyl carbamate, aryl carbamate, alkyl aryl carbamate,
- 25 alkyl thiocarbamate, aryl thiocarbamate, alkylaryl thiocarbamate, alkyl dithiocarbamate, aryl dithiocarbamate, alkylaryl dithiocarbamate, bicarbonate, carbonate, perchlorate, chlorate, chlorite, hypochlorite, perbromate, bromate, bromite, hypobromite,
- 30 tetrahalomanganate, tetrafluoroborate, hexafluoroantimonate, hypophosphite, iodate, periodate, metaborate, tetraaryl borate, tetra alkyl borate, tartrate, salicylate, succinate, citrate, ascorbate, saccharinate, amino acid, hydroxamic acid, thiotosylate,
- 35 and anions of ion exchange resins, or the corresponding anions thereof, or X, Y and Z are independently attached

-101-

to one or more of the "R" groups and n is an integer from 0 to 3;

M is a paramagnetic metal selected from the group consisting of metals having atomic numbers 21-29, 42-44

5 and 57-71; and

at least one of R_{10} , R_{11} , R_{12} , R_{13} , and R_{14} is other than hydrogen.

14. The compound of Claim 13 wherein M is Mn(II) or Gd(III).

10 15. The compound of Claim 13 wherein at least three of R , R' , R_1 , R'_1 , R_2 , R'_2 , R_3 , R'_3 , R_4 , R'_4 , R_5 , R'_5 , R_6 , R'_6 , R_7 , R'_7 , R_8 , R'_8 , R_9 , and R'_9 are other than hydrogen.

16. The compound of Claim 13 wherein at least one
15 of R_1 or R'_1 and R_2 or R'_2 , R_3 or R'_3 , and R_4 or R'_4 , R_5 or R'_5 , and R_6 or R'_6 , R_7 or R'_7 , and R_8 or R'_8 , and R_9 or R'_9 , and R or R' together with the carbon atoms to which they are attached form a saturated cyclic having 5 to 8 carbon atoms; and all of the remaining "R" groups are
20 independently selected from hydrogen, alkyl, or alkyl substituted with $-OR_{15}$ or $-NR_{15}R_{16}$, wherein R_{15} and R_{16} are independently hydrogen or alkyl.

17. The compound of Claim 13 wherein at least two
25 of R , R' , R_1 , R'_1 , R_2 , R'_2 , R_3 , R'_3 , R_4 , R'_4 , R_5 , R'_5 , R_6 , R'_6 , R_7 , R'_7 , R_8 , R'_8 , R_9 , and R'_9 are alkyl or alkyl substituted with $-OR_{15}$ or $-NR_{15}R_{16}$, wherein R_{15} and R_{16} are independently hydrogen or alkyl.

INTERNATIONAL SEARCH REPORT

Inter. Application No
PCT/US 95/03763

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K49/00 A61K51/04 C07F13/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 296 522 (DOW CHEMICAL CO) 28 December 1988 see claims ---	9-12
X	EP,A,0 287 465 (GUERBET SA) 19 October 1988 see page 8, line 56 - page 9, line 21; claims ---	1-5,7, 13-17
X	DE,A,24 61 919 (DU PONT) 17 July 1975 see page 6; figure X; table I see page 1, paragraph 1 ---	13
X	WO,A,92 04919 (MALLINCKRODT MEDICAL INC) 2 April 1992 see claims ---	1-5, 13-17
	--- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *Z* document member of the same patent family

Date of the actual completion of the international search

7 July 1995

Date of mailing of the international search report

01.08.95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

BERTE, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/03763

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 21017 (UNGER EVAN C ;SHEN DEKANG (US)) 26 November 1992 see page 5; figure III see claims 42,50,77,86 ---	1-5, 13-17
P,X	TETRAHEDRON LETT. (1994), 35(22), 3687-90 CODEN: TELEAY;ISSN: 0040-4039, ASTON, KARL W. ET AL 'Asymmetric synthesis of highly functionalized polyazamacrocycles via reduction of cyclic peptide precursors' see the whole document ---	13-17
X,P	TETRAHEDRON LETT. (1994), 35(6), 853-6 CODEN: TELEAY;ISSN: 0040-4039, 4 LENNON, PATRICK J. ET AL 'New conformationally constrained polyazamacrocycles prepared via the bis(chloroacetamide) method' see page 853, paragraph 1; figures 17,18 ---	5-7, 13-17
X	EP,A,0 524 161 (MONSANTO CO) 20 January 1993 see page 2, line 54 - page 4, line 15; claims ---	13-17
A	WO,A,82 04252 (BORREGAARD IND ;DALE JOHANNES (NO); BUEN SOLFRID (NO); KRANE JOST) 9 December 1982 ---	1-17
X	FR,A,2 246 555 (DU PONT) 2 May 1975 see claims 1,9,10 ---	13-17
P,A	WO,A,94 15925 (MONSANTO CO) 21 July 1994 see claims ---	1-17
X	CHEMICAL ABSTRACTS, vol. 110, no. 16, 17 April 1989, Columbus, Ohio, US; abstract no. 146606, NEWTON, JAMES E. ET AL 'Synthesis and characterization of the manganese(II) complex of [15]aneN5' see abstract & J. COORD. CHEM. (1988), 19(1-3), 265-77 CODEN: JCCMBQ;ISSN: 0095-8972, ---	13,14
A	PATENT ABSTRACTS OF JAPAN vol. 15, no. 461 (C-0887) 22 November 1991 & JP,A,03 197 468 (TEIJIN LTD) 28 August 1991 see abstract ---	1-17
-/--		

INTERNATIONAL SEARCH REPORT

Inter. Application No
PCT/US 95/03763

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>PATENT ABSTRACTS OF JAPAN vol. 12, no. 219 (C-506) 22 June 1988 & JP,A,63 014 780 (TOSOH CORP.) 21 January 1988 see abstract</p> <p>-----</p>	1-17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/03763

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-12 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 1-5, 7, 9-17
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
please see enclosure ../..!
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Incomplete search,...

II. Obscurities,

In view of the definition of products by means of their biological, chemical and/or pharmacological properties, the search has to be restricted for economic reasons.

The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims or examples.

(See Guidelines, Part B, Chapt. III, paragraph 3.6)

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/03763

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0296522	28-12-88	US-A- 4994560	19-02-91
		AU-B- 614973	19-09-91
		AU-A- 1833288	05-01-89
		CN-B- 1022839	24-11-93
		IL-A- 86835	25-01-94
		JP-A- 1026586	27-01-89
		NO-A- 951117	27-12-88
		US-A- 5006643	09-04-91
		US-A- 5064956	12-11-91
		US-A- 5284644	08-02-94
EP-A-0287465	19-10-88	FR-A- 2614020	21-10-88
		AU-B- 606146	31-01-91
		AU-A- 1461188	20-10-88
		CN-B- 1022411	13-10-93
		DE-A- 3877799	11-03-93
		ES-T- 2053779	01-08-94
		FI-B- 93830	28-02-95
		JP-A- 1211573	24-08-89
		NO-B- 176839	27-02-95
		US-A- 5417960	23-05-95
		US-A- 5049667	17-09-91
		ZA-A- 8802552	27-09-88
DE-A-2461919	17-07-75	US-A- 3930867	06-01-76
		BE-A- 824164	07-07-75
		GB-A- 1487261	28-09-77
WO-A-9204919	02-04-92	US-A- 5162109	10-11-92
		AU-A- 8851591	15-04-92
		CA-A- 2068424	14-03-92
		EP-A- 0500919	02-09-92
		JP-T- 5503107	27-05-93
WO-A-9221017	26-11-92	AU-B- 660033	08-06-95
		AU-A- 1998792	30-12-92
		CA-A- 2102605	24-11-92
		EP-A- 0594640	04-05-94
		JP-T- 6507904	08-09-94
		US-A- 5312617	17-05-94

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/03763

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0524161	20-01-93	AU-A- 2338392	23-02-93
		EP-A- 0598753	01-06-94
		JP-T- 6509566	27-10-94
		WO-A- 9302090	04-02-93
		ZA-A- 9205139	26-04-93
WO-A-8204252	09-12-82	EP-A, B 0080478	08-06-83
FR-A-2246555	02-05-75	US-A- 4001212	04-01-77
		CA-A- 1055022	22-05-79
		DE-A- 2447279	10-04-75
		GB-A- 1483821	24-08-77
		JP-A- 50059385	22-05-75
		US-I- B403326	23-03-76
WO-A-9415925	21-07-94	AU-B- 5964894	15-08-94